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A PHYLOGENETIC ANALYSIS OF ALYXIEAE (APOCYNACEAE) BASED ON *RBC*L, *MAT*K, *TRN*L INTRON, *TRN*L-F SPACER SEQUENCES, AND MORPHOLOGICAL CHARACTERS[1]

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Abstract

Within Rauvolfioideae (Apocynaceae), genera have long been assigned to tribes based mainly on only one or two superficial fruit and seed characters. Taxa with drupaceous fruits were included in Alyxieae. To elucidate relationships within Alyxieae, we analyzed phylogenetically a data set of sequences from four plastid DNA regions (*rbc*L, *mat*K, *trn*L intron, and *trn*L-F intergenic spacer) and a morphological data set for 33 genera of Apocynaceae, including representatives of all genera previously included in Alyxieae and two non-Apocynaceae species. Results of parsimony analysis indicate that Alyxieae as previously delimited are polyphyletic, with most genera falling into two main clades. The *Alyxia* clade includes seven genera: *Alyxia* Banks ex R. Br., *Lepinia* Decne., *Lepiniopsis* Valeton, *Pteralyxia* K. Schum., and *Condylocarpon* Desf. together with *Plectaneia* Thouars. (earlier included in Plumerieae) and *Chilocarpus* Blume (earlier included in Chilocarpeae). The *Vinca* clade includes eight genera: *Cabucala* Pichon, *Petchia* Livera, *Rauvolfia* L., *Catharanthus* G. Don, *Vinca* L., *Neisosperma* Raf., *Ochrosia* Juss., and *Kopsia* Blume. *Vallesia* Ruiz & Pav. and *Anechites* Griseb. are not related to either clade and come out as sister to *Aspidosperma* Mart. & Zucc. (Aspidospermeae) and *Thevetia* L. (Plumerieae), respectively. The fruit and seed

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† Siwert Nilsson passed away unexpectedly before the manuscript was completed. We lost both an excellent collaborator and a dear friend. We dedicate this paper to him.

characters previously used to demarcate Alyxieae are homoplasious, as are other morphological characters such as style head structure and syncarpy versus apocarpy. Conversely, pollen morphology, which has not previously played much of a role in tribal delimitation, was shown to be the most useful morphological character for delimiting Alyxieae from other tribes of Rauvolfioideae.

Key words: Alyxieae, Apocynaceae, classification, *mat*K, morphology, phylogeny, pollen, *rbc*L, systematics, *trn*L, *trn*L-F.

Tribal delimitation in Rauvolfioideae (usually referred to as Plumerioideae in the older literature) has previously been based on fruit and seed characters (Schumann, 1895; Pichon, 1949a, 1949c; Lý, 1986; Leeuwenberg, 1994a). One reason that fruit and seed characters have been so frequently used in classifications and keys is that they are readily observed, permitting easy recognition of many genera. The other main reason for the fruit-based tribal classifications is that the flowers of many Rauvolfioideae tend to be superficially similar. Many are relatively small with a whitish, salverform corolla, and there are few obvious distinguishing floral characters useful for differentiating tribes in this subfamily. The most detailed studies of Rauvolfioideae were those of Pichon (1948a, 1948b, 1949a, 1950b), who published extensively on the family. His classification was a great improvement over its predecessors. Its main weakness was that tribal delimitation was based mainly on a single fruit character. He split the rauvolfioid tribes into two main groups, depending on whether the deeper layers of the mesocarp were fleshy or dry. The group with a fleshy inner mesocarp was divided into five tribes: Carisseae, Ambelanieae, and Macoubeeae comprised those taxa with indehiscent berries and Chilocarpeae and Tabernaemontaneae included those taxa with fruit consisting of dehiscent follicles with arillate seeds. In the group with a dry mesocarp, he recognized three tribes. Two of them are characterized by dehiscent fruits: Alstonieae (Plumerieae sensu Leeuwenberg, 1994a), in which the fruit consists of a pair of follicles, and the monotypic Allamandeae, in which the fruit is a spiny unilocular capsule. The last tribe, Rauvolfieae (Alyxieae sensu Leeuwenberg, 1994a), contained all taxa in which the fruit is an indehiscent drupe with a stony endocarp. It is the relationships within this group that are the focus of this paper.

Pichon (1949a) recognized five subtribes within his Rauvolfieae: Rauvolfinae (including *Cabucala* Pichon, *Petchia* Livera, *Rauvolfia* L., and *Podochrosia* Baill.), Alyxiinae (including *Alyxia* Banks ex R. Br., *Lepinia* Decne., and *Lepiniopsis* Valeton), Ochrosiinae (including only *Ochrosia* Juss.), Vallesiinae (including *Vallesia* Ruiz & Pav. and *Kopsia* Blume), and Condylocarpinae (including *Rhipidia* Markgr. and *Condylocarpon* Desf.). Pichon included two genera as incertae sedis: *Anechites* Griseb. and *Notonerium* Benth. *Notonerium* has since been shown to belong to Boraginaceae (Crisp, 1983).

In his classification from 1994a, Leeuwenberg maintained Pichon's (1949a) circumscription of Rauvolfieae as well as the five subtribes included there but gave no insight into the delimitation of the subtribes, stating only that the relatively slight differences between its subtribes are not easily described in a concise way. The only differences between Leeuwenberg's (1994a) and Pichon's (1949a) tribal circumscriptions are that Leeuwenberg changed the name of the tribe to Alyxieae, put *Podochrosia* into synonymy under *Rauvolfia* and *Rhipidia* in *Condylocarpon* (following Fallen, 1983b), and included *Anechites* in Condylocarpinae, although Fallen (1983a) had suggested earlier that a position closer to taxa previously included in Cerbereae (*Cameraria* L., *Cerbera* L., *Cerberiopsis* Vieill. ex Pancher & Sebert, *Thevetia* L., and *Skytanthus* Meyen) was more appropriate. More recently, Leeuwenberg (1997) put *Cabucala* into synonymy under *Petchia*.

In addition to the taxa mentioned above, there are four other genera characterized by drupaceous fruits: *Cerbera*, *Thevetia*, *Cerberiopsis*, and *Cameraria*. These, together with *Skytanthus*, with follicular fruits, were split out of Rauvolfioideae and treated by Pichon (1948b) as a separate subfamily, Cerberoideae. The characters he used for delimitation of this subfamily are ambiguous. Leeuwenberg (1994a) recognized the group as defined by Pichon, but at the tribal level as Cerbereae. Morphological studies by Fallen (1985) suggested a close relationship between Cerbereae and *Allamanda* L., the sole genus placed in Allamandeae by Pichon (1949a) and Leeuwenberg (1994a). Studies based on molecular or combined morphological and molecular data (Endress et al., 1996; Sennblad & Bremer, 1996, 2000, 2002) indicated that the genera previously included in Cerbereae do form a natural group (see Potgieter & Albert, 2001, for a different opinion) and that they are only a part of a larger group that includes not only *Allamanda*, but also *Plumeria* L. (usually included in the Plumerieae: Rauvolfioideae). An analysis of Cerbereae is not the aim of this study, although some representatives from that tribe are included in our analyses.

Using fruit characters to delimit tribes in Rauvolfioideae is appealing because it allows taxa to be easily categorized and keys to be constructed.

However, other characters of these taxa do not indicate the same patterns of relationships. Phylogenetic analyses of mainly molecular data have shown that these fruit- and seed-based classifications are considerably more artificial than previously suspected. An *rbc*L analysis by Sennblad and Bremer (1996) indicated that *Catharanthus* G. Don (with dry dehiscent follicles and included in Plumerieae) was more closely related to taxa previously placed in Alyxieae sensu Leeuwenberg (1994a) than to other Plumerieae. In larger studies (Sennblad, 1997; Sennblad & Bremer, 2000, 2002), *Catharanthus* and *Vinca* L. formed a well-supported clade together with *Rauvolfia*, *Ochrosia*, and *Kopsia*, which have fleshy drupes. In the same study, *Chilocarpus* Blume, which has always been thought to be most closely related to Carisseae, formed a strongly supported clade with *Alyxia* and *Lepinia* (Alyxieae), confirming results of a strongly supported *Chilocarpus*–Alyxieae clade reported previously by Civeyrel (1996) and van der Ham et al. (2001). Pichon (1949a) already realized that *Geissospermum* Allemão (with indehiscent fruits and seeds embedded in pulp) is probably the nearest relative of *Aspidosperma* Mart. & Zucc. (with dry dehiscent follicles and wind-dispersed seeds with a diaphanous wing), a position supported by Potgieter and Albert (2001) and Simões et al. (2007). In addition, Potgieter and Albert (2001) found that *Vallesia* (with drupaceous fruits and seeds embedded in juicy pulp) is closely related to *Haplophyton* A. DC. (with dry dehiscent follicles and wind-dispersed comose seeds). Such results indicate that fruit characters in Apocynaceae are evolutionarily plastic in response to selective pressures for adaptations associated with wind or animal dispersal.

The most recent classifications of Apocynaceae s.l. (Endress & Bruyns, 2000; Sennblad & Bremer, 2002) attempted to rectify some of these anomalies. In the classification of Endress and Bruyns (2000), *Anechites* was moved to a newly defined Plumerieae (including Cerbereae sensu Leeuwenberg (1994a) as well as *Allamanda*), a position suggested by Fallen (1983a), and *Vallesia* was included with *Aspidosperma*, *Geissospermum*, and *Haplophyton* in a newly defined Alstonieae. The remainder of Alyxieae (sensu Leeuwenberg, 1994a) was split into two tribes, Alyxieae and Vinceae, based on molecular results as well as additional morphological characters. Alyxieae sensu Endress and Bruyns (2000) included seven genera: *Alyxia*, *Pteralyxia* K. Schum., *Lepinia*, *Lepiniopsis*, *Plectaneia* Thouars, *Condylocarpon*, and *Chilocarpus*. Vinceae included *Amsonia* Walter, *Catharanthus*, *Vinca*, *Rauvolfia*, *Petchia*, *Kopsia*, *Neisosperma* Raf., and *Ochrosia*. *Rhazya* Decne. was considered to be synonymous with *Amsonia*, and *Cabucala* with *Petchia* (following Leeuwenberg, 1997). Simões et al. (2007) treated *Amsonia* as a genus incertae sedis and transferred *Laxoplumeria* Markgr., *Tonduzia* Pittier, and *Kamettia* Kostel. to Vinceae, bringing the total number of genera in the tribe up to 10.

The classification of Sennblad and Bremer (2002) proposed a new system that is compatible with traditional Linnaean nomenclature but uses a variant of the definitions used in phylogenetic nomenclature to improve the stability of classifications. Although they do not provide lists of included genera, their definitions of the tribes containing traditional Alyxieae genera are completely congruent with those of Endress and Bruyns (2000). The aim of this study is to cladistically evaluate Alyxieae and Vinceae as circumscribed by Endress and Bruyns (2000) in comparison with previous classifications, to re-examine the usefulness of fruit and seed characters for tribal delimitation within Rauvolfioideae, and to discover new morphological characters that have hitherto received little attention in classification of this subfamily but show phylogenetic potential.

Materials and Methods

Taxon Sampling

The ingroup taxa were chosen to include representatives of all genera of Alyxieae (sensu Leeuwenberg, 1994a), as well as other putatively related genera. The outgroup taxa are one genus each of Loganiaceae and Gelsemiaceae, which several studies (Bremer & Struwe, 1992; Chase et al., 1993; Savolainen et al., 2000; Soltis et al., 2000) have demonstrated to be closely related to Apocynaceae (Appendices 1, 2). Other more narrowly focused studies on Gentianales (Struwe et al., 1994; Endress et al., 1996; Backlund et al., 2000) have also indicated that Loganiaceae and Gelsemiaceae are the closest families to Apocynaceae. For the morphological analyses, we omitted the outgroups altogether because, in preliminary analyses, one or the other of these genera was embedded in different portions of the ingroup due to obvious parallelisms of certain characters; we arranged the morphological tree with the same group sister to the rest as in the molecular results.

Floral Structure

Fixed flowers at or near anthesis (only buds were available for *Lepiniopsis*) were dehydrated in an alcohol-xylene series, embedded in paraplast, cut with a rotary microtome at 10 μm, and stained with safranin and astra blue. For SEM studies, material was critical-point dried and then sputter-coated with gold.

POLLEN MORPHOLOGY

Pollen material was sampled from the following herbaria: BISH, BR, COL, G, L, P, PTBG, QCA, S, UB, WAG, and Z. Pollen studies were carried out in Leiden and Stockholm. For light microscopy (LM), pollen material was acetolyzed (except for *Condylocarpon* and *Vinca*), mounted in glycerine jelly, and sealed with paraffin. Generally, 10 pollen grains were measured for polar axis (P) and equatorial diameter (E). For SEM, pollen was sputter-coated with gold and examined with a JSM 5300 or JSM 6300 scanning electron microscope (JEOL, Tokyo). Frozen sections were made using an Ames Tissue-TEK Cryostat. For transmission electron microscopy (TEM), unacetolyzed material (whole anthers) was embedded in Spurr resin or 3/7 Epon, sectioned with a LKB Ultrotome III or V, poststained with uranylacetate and lead citrate, and examined with a Zeiss 10, a JEOL 100-S, or a JEM 1010. Terminology is according to Punt et al. (1994).

OTHER MORPHOLOGICAL AND CHEMICAL CHARACTERS

Information on fruit and seeds were taken from observations of herbarium specimens as available. Several fruit and seed characters were taken from the literature, as were data on the presence of laticifers and intraxylary phloem (Solereder, 1892; Schumann, 1895; Valeton, 1895; Degener, 1946; Pichon, 1947a, 1947b, 1948a, 1948b, 1948c, 1948d, 1949a, 1949b, 1949c, 1950a, 1950b, 1950c, 1952; Gensel, 1969; Markgraf, 1971, 1976, 1979; Markgraf & Huber, 1975; Corner, 1976; Conn, 1980; Leeuwenberg & Leenhouts, 1980; Rogers, 1986; Rudjiman, 1986; Pagen, 1987; Metcalf & Chalk, 1989; Rosatti, 1989; Wagner et al., 1990; Sévenet et al., 1994; Forster & Williams, 1996; Omino, 1996; Leeuwenberg, 1997; Sidiyasa, 1998; Lin & Bernardello, 1999). Chemical data were taken from the literature (Johns et al., 1968; Hegnauer, 1970, 1989; Coppen & Cobb, 1983; Kisakürek et al., 1983; Homberger & Hesse, 1984; Bisset, 1987; Endress et al., 1990; Wagner et al., 1990; Zhu et al., 1990; Attaurrahman et al., 1989, 1991; Arambewela & Ranatunge, 1991; Jensen, 1992; Sévenet et al., 1994; Zeches et al., 1995; Kam et al., 1997).

MOLECULAR METHODS

Nine new sequences of *rbc*L, 16 of *mat*K, and 11 of the *trn*L intron and *trn*L-F intergenic spacer were produced for this study; the other sequences were published previously (Appendix 2). Total DNA was extracted from fresh leaves, silica gel–dried material (Chase & Hills, 1991), or herbarium material using the methods of Saghai-Maroof et al. (1984) or modified Doyle and Doyle (1987). For the latter, DNA samples were purified by ultracentrifugation in CsCl–ethidium bromide gradients (1.55 g/ml). Additional purification using the QIAquick PCR purification kit (Qiagen, Valencia, California) was performed in cases with problematic polymerase chain reaction (PCR) amplification using the manufacturer's protocol. Double-stranded DNA was amplified with PCR primers for *rbc*L from Fay et al. (1998); the *trn*L intron and *trn*L-F intergenic spacer (hereafter, *trn*L-F) were amplified using the c and f primers of Taberlet et al. (1991); *mat*K primers were those of Endress et al. (1996) and Johnson and Soltis (1994). Direct sequencing of PCR products was performed using the PCR primers plus internal sequencing primers. For *rbc*L, the internal primers were those of Fay et al. (1998); for *trn*L-F, we used the d and e primers of Taberlet et al. (1991); and for *mat*K, we designed two new internal primers: 734F, 5′-ATGTATGTGACTACGAATCA-3′ and 829R, 5′-ACTTTCTATTTTTCCATAGA-3′. In a number of cases, we also used the internal sequencing primers as PCR primers to amplify shorter products. For sequencing, we used either the Dye Deoxy Terminator Cycle Sequencing or Big Dye kits of Applied Biosystems (ABI; Warrington, Cheshire, United Kingdom). Sequencing reactions were carried out directly on the cleaned PCR products and run on an ABI 277a automated sequencer at Kew following the manufacturer's protocols.

CLADISTIC ANALYSES

The data matrix comprised four submatrices: each of the three DNA regions plus morphology. The morphological submatrix (Appendix 3) comprises 54 characters from floral, fruit, vegetative, and pollen morphology and phytochemistry (Appendix 4). All analyses were performed using PAUP* 4.0b10 (Swofford, 2002). Heuristic searches were performed with all characters given unit weight (Fitch parsimony; Fitch, 1971), and each submatrix was analyzed separately before their joint combined analysis. Each of the searches used the following settings: 1000 replicates of random taxon entry, the subtree pruning re-grafting (SPR) branch swapping algorithm, and MULTREES on (saving multiple equally parsimonious trees) but holding only 10 trees per replicate. All the shortest trees were then collected and used as starting trees for a search with a 25,000-tree limit. If the tree limit was reached, then swapping was allowed to continue until all 25,000 trees were swapped to completion.

Bootstrap percentages (BP) (Felsenstein, 1985) were calculated with 500 replicates on each of the submatrices as well as on the two combined matrices

(molecular combined and molecular/morphological combined). We used the following settings: SPR branch swapping and MULTREES on, holding only 10 trees per step. All other settings were those of the standard defaults of PAUP* 4.0. This strategy produces results statistically indistinguishable from other, more thorough bootstrap protocols (Salamin et al., 2003). We apply the following arbitrary scale in our discussion: 50%–74%, weakly supported; 75%–84%, moderately supported; 85%–100%, strongly supported. Alignment for *rbc*L and *mat*K was a simple matter; the former had no length variation, and the latter had only a few easily characterized insertions/deletions (indels). For *trn*L-F, we started with the alignment of Potgieter and Albert (2001) and added the additional taxa needed for this analysis, which required adding a few more insertions; we did adjust their alignment in places following Kelchner (2000). We analyzed the *trn*L intron and *trn*L-F intergenic spacer in a single analysis (which can be considered "non-coding" because there is only about a 30 bp region of the *trn*L exon included); this region, termed *trn*L-F, is composed of two unrelated parts, but the number of variable sites is the lowest and, even when combined, these produced a highly unresolved strict consensus tree. We do report statistics for these two regions separately (Appendix 2) but consider results only for the two combined.

Incongruence of different regions of plastid DNA would be unexpected because recombination is unknown in the generally uniparentally inherited plastid genome. Several tests for combinability have been developed, but we have not used any of them in this paper. Such tests have proven to be unreliable indicators of incongruence (Reeves et al., 2001), so we attach no particular significance to results of these tests but instead prefer to look for cases of strongly supported and incongruent patterns of relationships. Differences in relationships are to be expected with different matrix components simply due to sampling effects where there are too few variable characters to obtain clear patterns. If, however, there are only a few characters in a matrix, such as is the case here with the morphological characters, differentiating between sampling error and incongruent patterns is extremely difficult. We note that the morphologically based estimates of relationships deviate from those based on DNA data, particularly for the Vinceae, but the small number of morphological characters (only 54) does not permit us to say whether the differences between molecular and morphological patterns are evidence of true incongruence.

Character state distributions of selected morphological characters were individually mapped onto the total evidence tree (Figs. 10, 11) using MacClade 4.0 (Maddison & Maddison, 2000) to illustrate character evolution and compare the usefulness of characters that have previously been used in delimitation of Alyxieae.

Results

Morphology

Analysis of the morphological matrix produced 31 trees in three islands (18, 4, and 9 trees) of equally parsimonious trees, each of 246 steps with a consistency index (CI) of 0.37 and a retention index (RI) of 0.62. A strict consensus tree of all three islands (Fig. 1; numbers below the branches are BPs) shows that the position of several genera is unclear with these morphological data, and the three islands place them in different relative positions to the clades that are consistently resolved in all three islands. The positions of *Vallesia antillana* Woodson, *V. glabra* (Cav.) Link, and the *Plumeria* clade (*Allamanda cathartica* L., *Anechites nerium* (Aubl.) Urb., *Plumeria rubra* L., and *Thevetia peruviana* (Pers.) K. Schum.) are consistent in all three islands, as is a clade composed of all remaining taxa. Within the last, *Acokanthera oblongifolia* (Hochst.) Codd, *A. oppositifolia* (Lam.) Codd, *Molongum laxum* (Benth.) Pichon, *Picralima nitida* (Stapf) T. Durand & H. Durand, *Pleiocarpa mutica* Benth., *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult., and *T. pandacaqui* Lam. occupy different positions in each of the three islands with respect to the two consistently resolved clades: (1) *Alstonia scholaris* (L.) R. Br. to *Rhazya stricta* Decne. and (2) *Alyxia oblongata* Domin and *A. ruscifolia* R. Br. to *Nerium oleander* L. (Fig. 1). The other conspicuously differently placed group is that composed of *Amsonia ciliata* Walter, *A. tabernaemontana* Walter, *Rhazya stricta*, and *Catharanthus roseus* (L.) G. Don + *Vinca major* L. and *V. minor* L., which in two of the islands (18 and 9 tree islands) are a clade but in the other island form a grade. Clades that receive moderate to strong BPs are the following: *Neisosperma nakaiana* (Koidz.) Fosberg & Sachet + *Ochrosia coccinea* (Teijsm. & Binn.) Miq. (BP 83), *Alyxia oblongata* and *A. ruscifolia* + *Lepinia marquisensis* Lorence & W. L. Wagner, *L. solomonensis* Hemsl. and *L. taitensis* Decne. + *Lepiniopsis ternatensis* Valeton and *L. trilocularis* Markgr. + *Pteralyxia kauaiensis* Caum and *P. macrocarpa* (Hillebr.) K. Schum. (BP 97; the last two genera with BP 96; the *Alyxia* clade), *Kibatalia gitingensis* (Elmer) Woodson + *Mascarenhasia arborescens* A. DC. + *Nerium oleander* (BP 98; the first two genera with BP 94), *Picralima nitida* + *Pleiocarpa mutica* (BP 84), and *Allamanda cathartica*

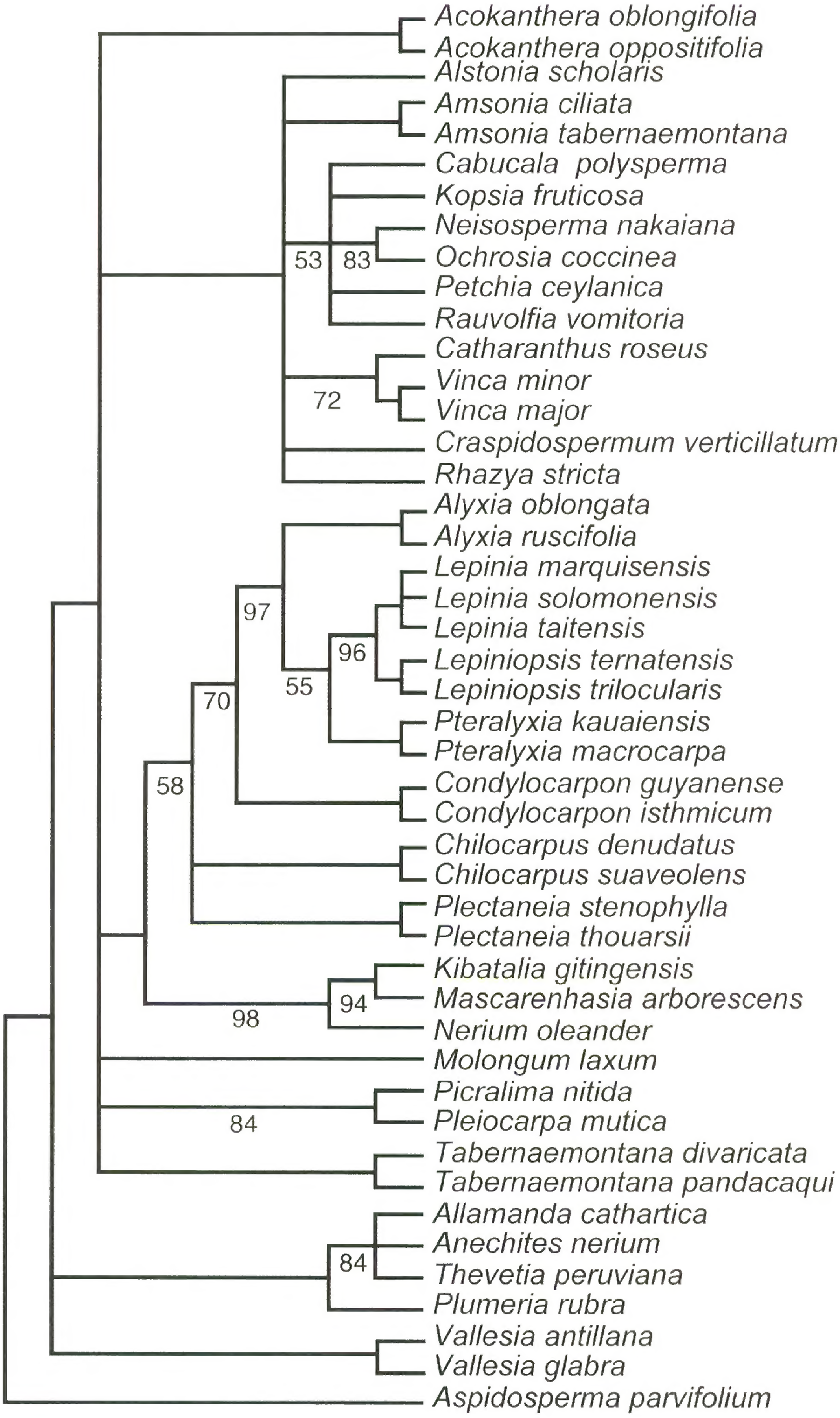

Figure 1. Strict consensus tree of the three islands found in the morphological analysis. Numbers below the branches indicate bootstrap percentages greater than 50%.

+ *Anechites nerium* + *Thevetia peruviana* (BP 84). *Catharanthus roseus* + *Vinca major* and *V. minor* nearly reached the moderate level (BP 72).

ANALYSIS OF *RBCL*

Of the 1398 included positions, 244 (18%) were variable and 146 (10%) were potentially parsimony informative. Analysis produced two islands of equally parsimonious trees, one of 39 trees and the other of 16 trees; they had 479 steps with CI (including uninformative positions) of 0.59 and RI of 0.63. The two islands differ in the relative positions of *Neisosperma oppositifolia* (Lam.) Fosberg & Sachet and *Rauvolfia mannii* Stapf and *Chilocarpus suaveolens* Blume + *Condylocarpon guyanense* Desf. In island one, the latter form a clade with the *Kibatalia gitingensis* + *Mascarenhasia arborescens* + *Nerium oleander* clade, and *Neisosperma oppositifolia* and *Rauvolfia mannii* are unresolved members of the clade including *Cabucala polysperma* (Scott-Elliott) Pichon + *Petchia ceylanica* (Wight) Livera + *Ochrosia coccinea*. In island two, *Neisosperma oppositifolia* + *Rauvolfia mannii* are sister to *Catharanthus roseus* + *Vinca minor*, and *Chilocarpus suaveolens* + *Condylocarpon guyanense* are unresolved. The strict consensus of both islands (Fig. 2) therefore shows these taxa to be unresolved. Supported clades that are also found in the morphological analysis include the following: *Allamanda cathartica* + *Plumeria inodora* Jacq. (BP 100) and *Anechites nerium* + *Thevetia peruviana* (BP 97; the whole *Plumeria* clade, BP 62); *Alyxia ruscifolia* + *Lepinia taitensis* + *Lepiniopsis trilocularis* + *Pteralyxia kauaiensis* (the *Alyxia* clade; BP 99); *Picralima nitida* sister to *Pleiocarpa mutica* (BP 100); *Catharanthus roseus* + *Vinca minor* (BP 68); and *Kibatalia gitingensis* + *Mascarenhasia arborescens* + *Nerium oleander* (the *Nerium* clade; BP 93). Well-supported clades that are not strongly in conflict with the morphological results include: *Aspidosperma triternatum* Rojas Acosta as sister to *Vallesia antillana* (BP 100); *Plectaneia stenophylla* Jum. as sister to the *Alyxia* clade (BP 98), within which the topology is the same as in the morphological results; *Amsonia tabernaemontana* as sister to *Rhazya stricta* (BP 100); *Cabucala polysperma* as sister to *Petchia ceylanica* (BP 100); and *Molongum laxum* as sister to *Tabernaemontana divaricata* (BP 95). *Chilocarpus suaveolens* as sister to *Condylocarpon guyanense* (BP 85) is contradicted in the morphological results by a weak BP 70 for the latter to be sister to *Alyxia* clade. Weakly supported results not contradicting relationships produced by morphology are the *Plumeria* clade (BP 62; *Allamanda cathartica*, *Anechites nerium*, *Plumeria inodora*, and *Thevetia peruviana*); *Picralima nitida* + *Pleiocarpa mutica* as sister to the *Alyxia* clade, plus *Plectaneia stenophylla* (BP 70); and the *Vinca* clade (BP 65; *Cabucala polysperma*, *Petchia ceylanica*, *Ochrosia coccinea*, *Catharanthus roseus*, *Vinca minor*, *Neisosperma oppositifolia*, and *Rauvolfia mannii*).

ANALYSIS OF *MATK*

The aligned *mat*K matrix contained 1647 bp, of which 561 (34%) were variable and 250 (15%) were potentially parsimony informative. We were unable to amplify the following taxa for *mat*K: *Anechites*, *Lepinia*, *Lepiniopsis*, *Ochrosia*, and *Plectaneia*. Analysis produced a single, most parsimonious tree of 970 steps with a CI of 0.73 and an RI of 0.59. Patterns of relationships are nearly identical to those found with *rbc*L, but in general, BPs are higher than with *rbc*L or *trn*L-F (Fig. 3). Patterns that were not observed with *rbc*L include: *Chilocarpus suaveolens* + *Condylocarpon guyanense* (BP 100) as sister (BP 99) to the *Alyxia* clade (BP 100); *Alstonia scholaris* strongly supported in an isolated position as sister (BP 93) to all but *Aspidosperma triternatum* + *Vallesia antillana*; and *Aspidosperma triternatum* + *Vallesia antillana* (BP 82) moderately supported as sister to the rest of the ingroup (BP 97).

ANALYSIS OF *TRNL*-F

The aligned *trn*L-F matrix consisted of 1206 bp (761 bp from the *trn*L intron and 445 bp from the *trn*L-F spacer). We were unable to amplify the following taxa for *trn*L-F: *Kibatalia* G. Don and *Lepinia*. Analysis produced over 25,000 trees of 460 steps with a CI of 0.78 and an RI of 0.70 (*trn*L intron: 292 steps with a CI of 0.76 and an RI of 0.69; *trn*L-F intergenic spacer: 168 steps with a CI of 0.82 and an RI of 0.72) (Fig. 4). Relationships from these two, largely non-coding regions are similar to those estimated from *rbc*L and *mat*K (Figs. 2, 3). The major noteworthy result (also observed in the *mat*K results, but which received BP < 50) is a strongly supported clade (BP 97) composed of *Nerium oleander* and *Mascarenhasia arborescens* of the *Nerium* clade, observed with morphology, *rbc*L, and *mat*K, with *Acokanthera oppositifolia* weakly supported as sister (BP 60) also observed but without support with *mat*K, and *Allamanda indet.* + *Plumeria alba* Kunth (BP 95), *Anechites nerium*, and *Thevetia ahouai* (L.) A. DC. (the last two unresolved with respect to the *Nerium* clade).

COMBINED MOLECULAR ANALYSES

The combined data set produced 46 equally parsimonious trees of 1925 steps with a CI of 0.70

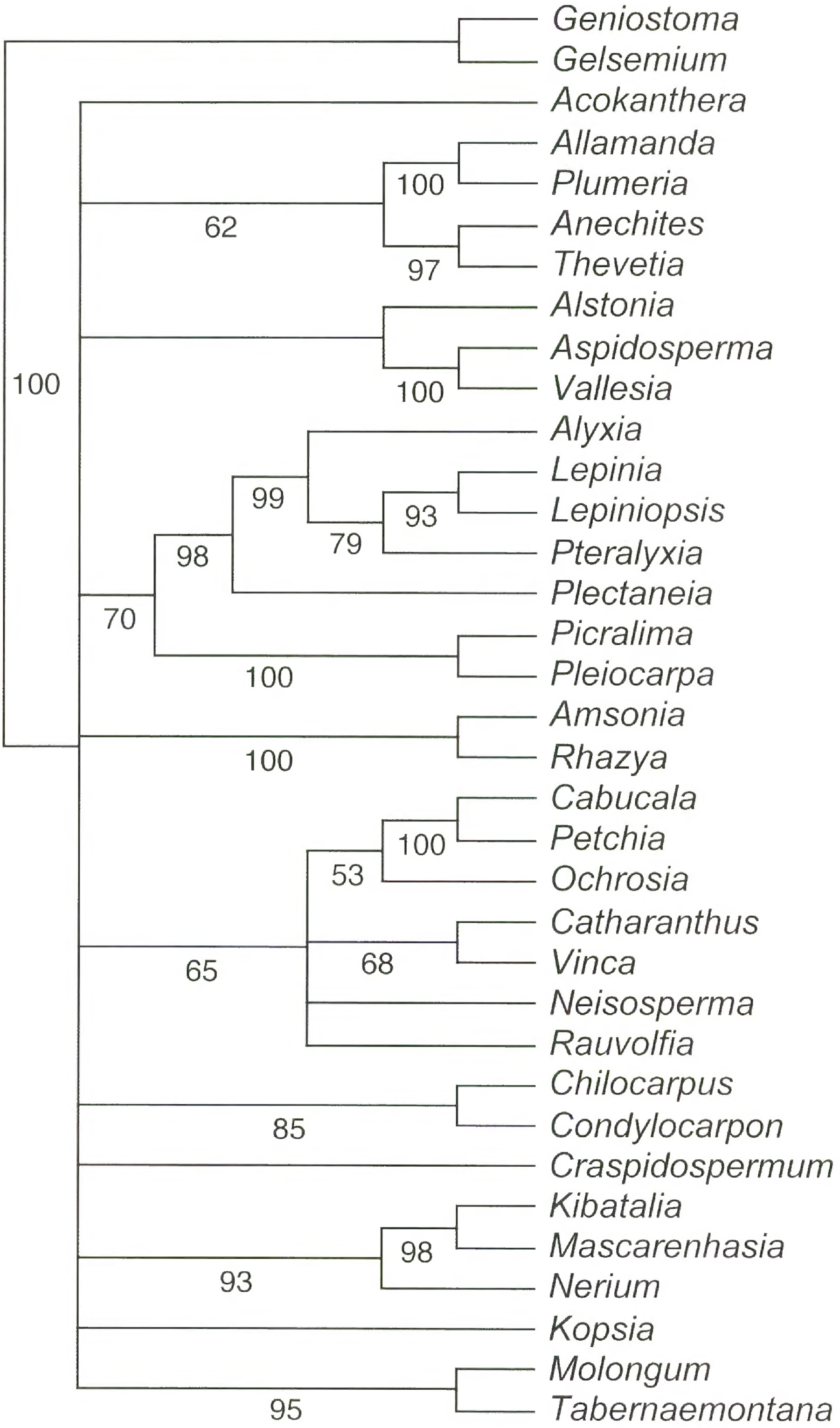

Figure 2. Strict consensus tree of the two islands found with the *rbc*L data. Numbers below the branches indicate bootstrap percentages greater than 50%.

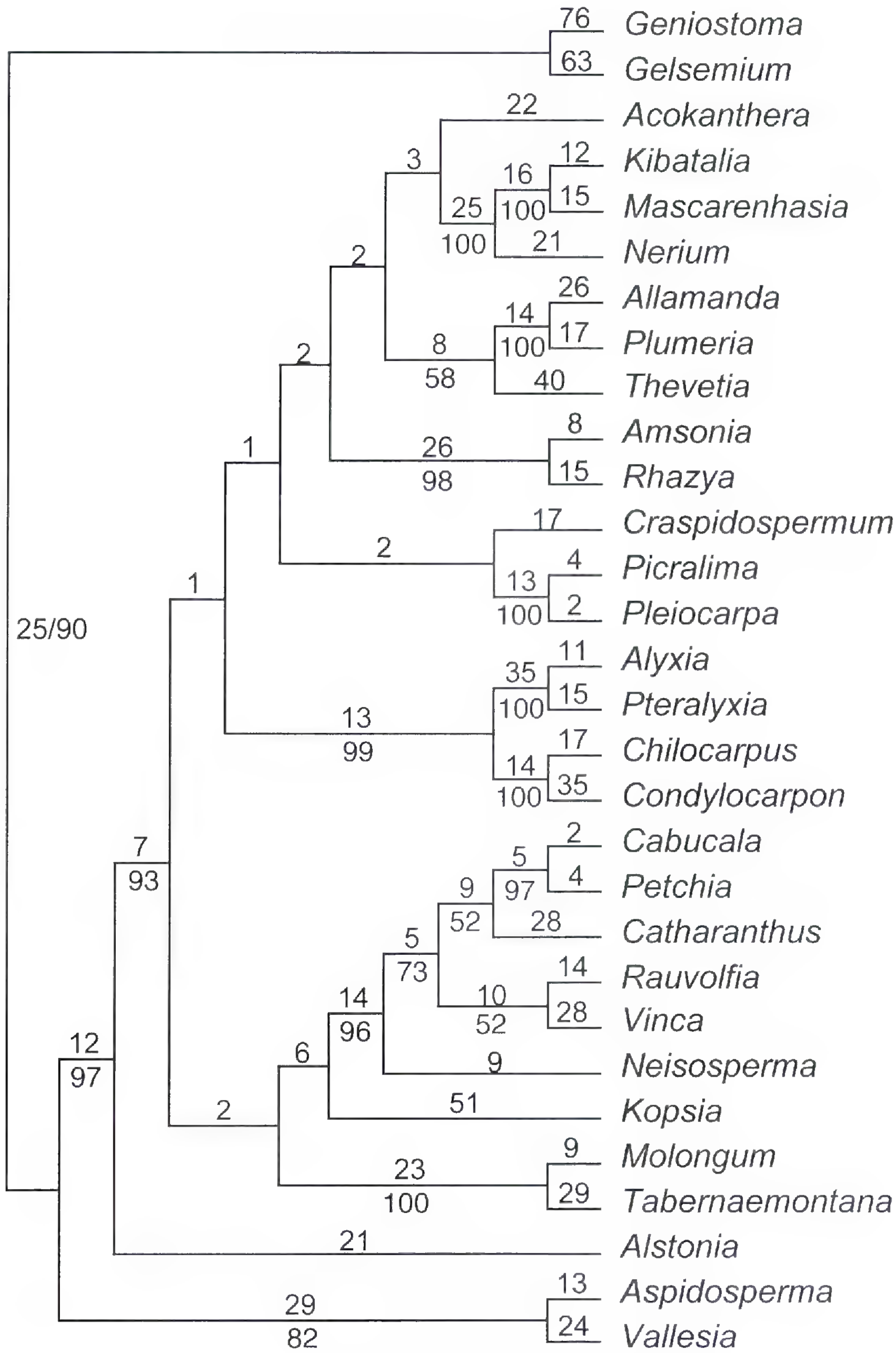

Figure 3. The single most parsimonious tree found with the *mat*K data. Numbers above the branches indicate estimated substitutions, ACCTRAN optimization. Numbers below the branches indicate bootstrap percentages greater than 50%.

and an RI of 0.62. One of the shortest individual trees is shown in Figure 5, with estimated substitutions (ACCTRAN optimization) indicated above the branches and BPs below; groups not present in all shortest trees are marked with an arrowhead. We show a single tree to illustrate relative levels of genetic divergence. The contribution of each region to this tree was: *rbc*L, 488 steps (vs. 479 for the *rbc*L trees); *mat*K, 974 steps (vs. 970 for the *mat*K tree); *trn*L intron, 293 steps (vs. 292 on the *trn*L-F trees); and *trn*L-F intergenic spacer, 170 steps (vs. 168 on the *trn*L-F trees). Patterns of relationships are much like those in the previous analyses, and BPs are generally higher than in any of the individual analyses.

ANALYSIS OF ALL DATA COMBINED

The combined data produced a single, most parsimonious tree of 2226 steps with a CI of 0.65 and an RI of 0.60 (Fig. 6). The DNA optimized onto

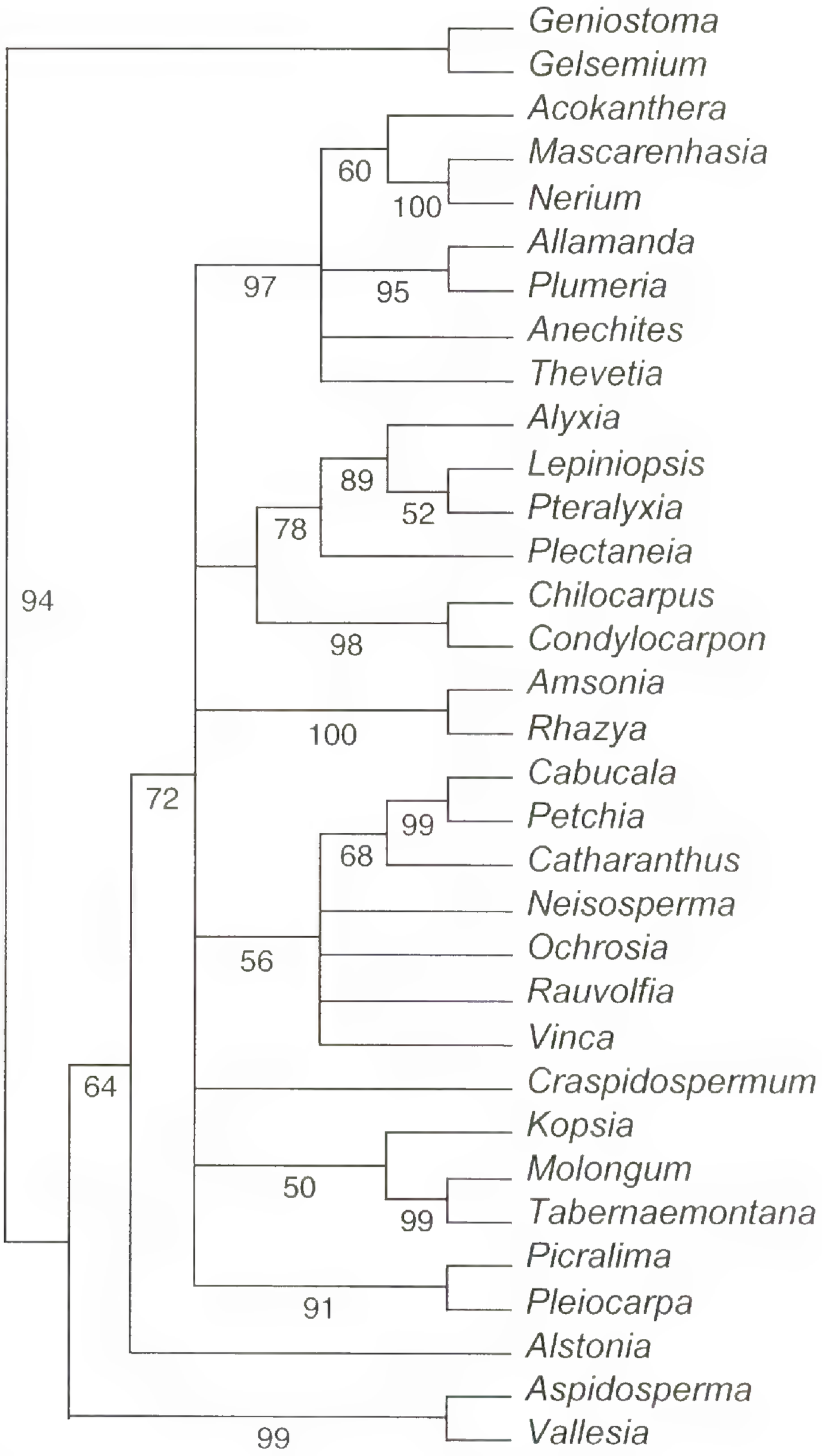

Figure 4. Strict consensus tree of the 25,000 equally most parsimonious trees found with the *trn*L-F data. Numbers below the branches indicate bootstrap percentages greater than 50%.

this tree is 1926 steps, one step longer than the combined DNA tree; this step is caused by shifting *Kopsia fruticosa* (Ker. Gawl.) A. DC. and *Molongum laxum* + *Tabernaemontana* (two spp., Appendix 2) from an unresolved position with respect to the major clades into positions as a grade with respect to the *Vinca* clade. Otherwise, relationships are exactly as with the combined molecular data. The morphological data optimized onto the combined trees (ACCTRAN optimization) was 276 steps with a CI of 0.33 and an RI of 0.55, versus 266 steps with a CI of 0.38 and an RI of 0.61 in the morphological analysis.

Discussion

Evaluation of Traditional Circumscriptions of Alyxieae

Both the morphological and the molecular analysis indicate that Alyxieae as previously circumscribed are polyphyletic. Of the individual data sets analyzed, the tree based on *mat*K provided the best support, followed by that of *trn*L-F. BPs in the tree based on *rbc*L and the morphological data set were low, with much of the tree a polytomy, and the positions of several genera were equivocal. Even then, however,

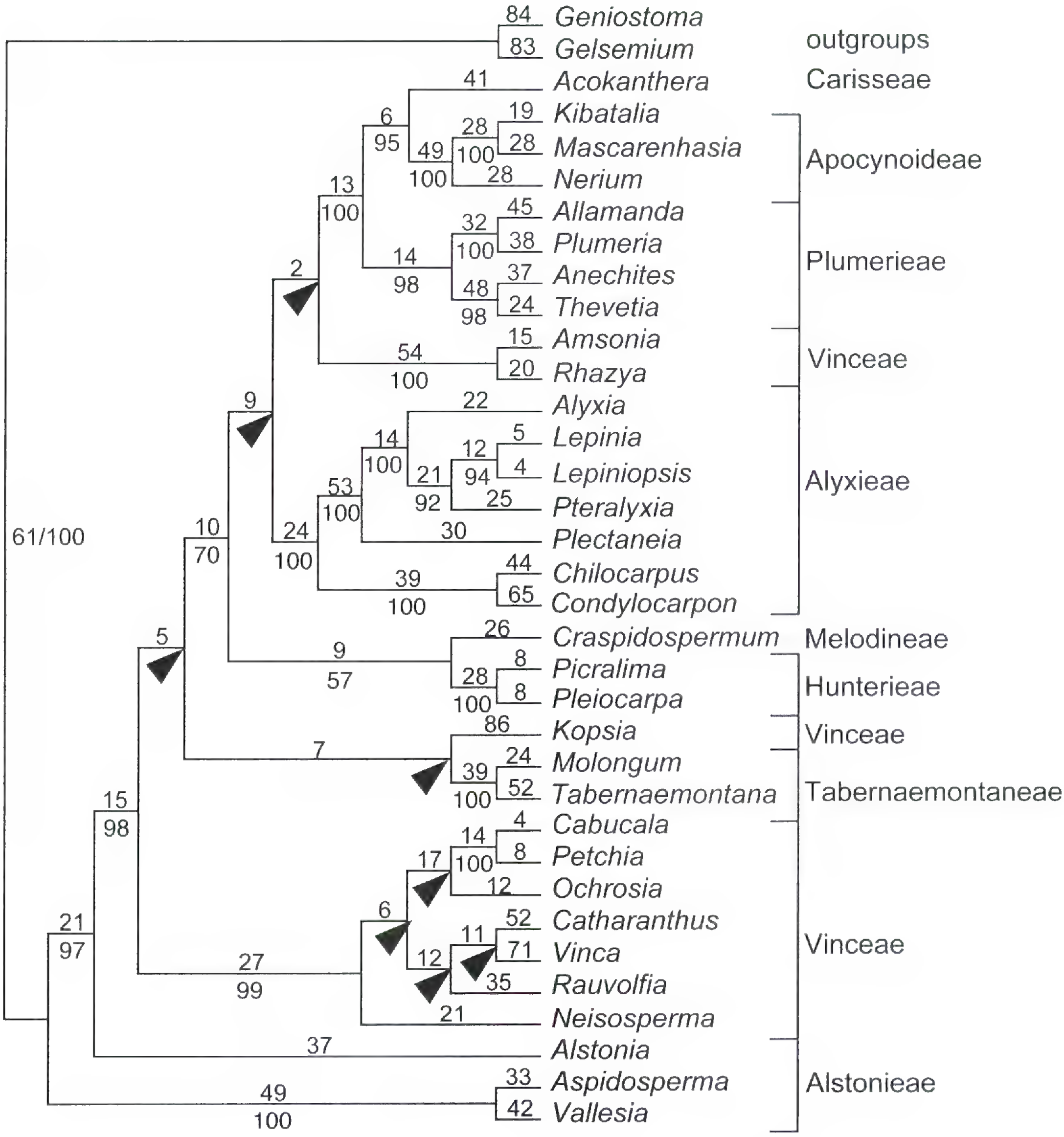

Figure 5. One of the most parsimonious trees found with the combined molecular data. Numbers above the branches indicate estimated substitutions, ACCTRAN optimization. Numbers below the branches indicate bootstrap percentages greater than 50%. Groups not present in all 46 shortest trees are noted with an arrowhead.

clusters of genera are present. The combined data tree is similar to that produced by the combined molecular data and provides much better support for patterns of relationship already seen in each of the individual trees. Therefore, the remainder of the discussion will be based on the total combined tree (Fig. 6). This tree is not intended to represent relationships within or among tribes other than Alyxieae and Vinceae.

All genera traditionally included in Alyxieae are preceded by a dot in Figure 6. *Vallesia* and *Anechites*, both included in Alyxieae by Pichon (1949a, 1950b, as Rauvolfieae) and maintained there by Leeuwenberg (1994a), are not closely affiliated with any of the other members of the ingroup. *Vallesia* is sister to *Aspidosperma*, a position that supports results of previous phylogenetic studies (Sennblad & Bremer, 2000, 2002; Potgieter & Albert, 2001; Simões et al., 2007). It is unlikely that a close relationship between *Vallesia* and *Aspidosperma* would have been predicted based on their floral or fruit structures; they are too plesiomorphic to be of much help, and the small indehiscent drupaceous fruits of the former look very

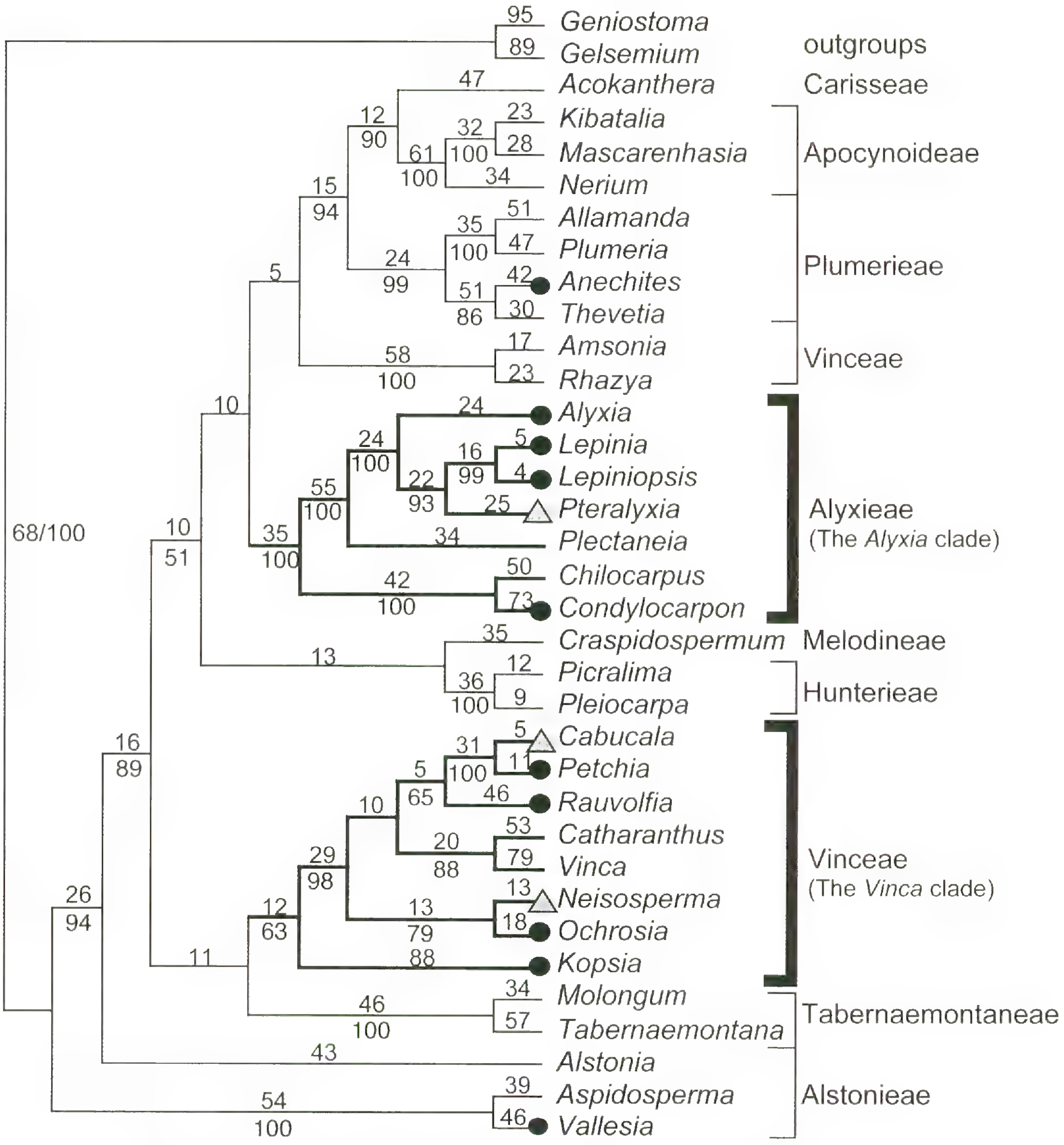

Figure 6. The single most parsimonious tree found in the total combined analysis of the morphological and molecular data. Numbers above the branches indicate estimated substitutions, ACCTRAN optimization. Numbers below the branches indicate bootstrap percentages greater than 50%. The *Alyxia* clade and the *Vinca* clade are indicated with thicker branches and brackets. • = Alyxieae (Rauvolfieae in earlier classifications). Δ = taxa not recognized at generic rank by Leeuwenberg (1994a). Tribal names on right follow the classification of Endress and Bruyns (2000).

different from the dehiscent follicular fruits of the latter. A close relationship between the two genera is, however, supported by pollen morphology. In both genera the pollen has five or six apertures (as opposed to the usual 3-aperturate condition), which are surrounded by distinctive prominent ridges. The inclusion of *Vallesia* in Alstonieae sensu Endress and Bruyns (2000) is supported by previous *rbc*L data (Sennblad & Bremer, 2000, 2002), but not by *trn*L-F data (Potgieter & Albert, 2001), which included more genera near the base of Apocynaceae. In our analysis, *Anechites* is sister to *Thevetia*, grouping with members of Cerbereae of traditional classifications (Cerberoideae of Pichon, 1948b), which confirms results based on morphology (Fallen, 1983a; Alvarado-Cárdenas & Ochoterena, 2007) and earlier *rbc*L data (Sennblad & Bremer, 2000, 2002) and supports Endress and Bruyns' (2000) inclusion of *Anechites* in Plumerieae. The remainder of the genera of Alyxieae fall into two main clades.

RELATIONSHIPS WITHIN VINCEAE AND ALYXIEAE

The first main cluster of Alyxieae in the combined analysis (Fig. 6) is the *Alyxia* clade and includes *Alyxia*, *Lepinia*, *Lepiniopsis*, and *Condylocarpon*. In addition, the placement of *Chilocarpus*, *Plectaneia*, and *Pteralyxia* in this clade is well supported. This corroborates the positions of *Alyxia*, *Lepinia*, and *Chilocarpus* in the study by Sennblad and Bremer (2000, 2002) based on *rbc*L data. Except for *Condylocarpon*, the members of this group have irregular pollen grains with relatively large porate apertures. The aperture number is usually two; *Lepinia* and *Lepiniopsis* have three or four apertures (van der Ham et al., 2001). Within the *Alyxia* clade, *Alyxia*, *Lepinia*, *Lepiniopsis*, *Pteralyxia*, and *Plectaneia* form a clade (Fig. 6). The first three genera correspond to Pichon's (1949a) and Leeuwenberg's (1994a) Alyxiinae. *Pteralyxia* was considered to be a synonym of *Alyxia* by Pichon (1949a) and a synonym of either *Alyxia* or *Ochrosia* by Leeuwenberg (see Gunn et al., 1992 and van der Ham et al., 2001: 169, 187). *Plectaneia*, in contrast, has previously been included in Plumerieae and has usually been considered to be related to genera such as *Gonioma* E. Mey., *Stephanostegia* Baill., and *Craspidospermum* Bojer ex A. DC. (Alstoniinae of Schumann, 1895; Plectaneiinae of Pichon, 1949a; Craspidosperminae of Leeuwenberg, 1994a), all of which are included in Melodineae in Endress and Bruyns (2000). In terms of pollen morphology, *Alyxia*, *Lepinia*, *Lepiniopsis*, and *Pteralyxia* form a tight-knit monophyletic group (Fig. 7). The porate apertures can be large (maximum 21–33 μm), their margins are clearly thickened (less clearly also in *Chilocarpus*), the inner exine surface is granular (also in subfamily Apocynoideae), the inner exine layer (nexine) is completely endexinous, the infratectum is hardly recognizable (being reduced to ± sparse gaps in the inner ectexine), and a relatively thick tectum is present (also in several other taxa). *Lepinia* and *Lepiniopsis* share the presence of an ornamentation consisting of anastomosed verrucae (van der Ham et al., 2001). In the large analysis of *trn*L-F by Potgieter and Albert (2001), *Alyxia*, *Lepiniopsis*, *Condylocarpon*, and *Plectaneia* were supported in Alyxieae, whereas *Pteralyxia* affined with Plumerieae. This is an unlikely position for *Pteralyxia* considering its distinctive pollen, which is a synapomorphy of Alyxieae and found nowhere else in Apocynaceae (van der Ham et al., 2001). As the same vouchered specimen was used as the source of DNA for both studies, the reason for this discrepancy between our *trn*L-F results and those of Potgieter and Albert (2001) is most likely due to a mix-up in the laboratory. It is noteworthy that of the seven genera in the Alyxieae, four, *Lepinia*, *Lepiniopsis*, *Pteralyxia*, and *Plectaneia*, are island endemics (Leeuwenberg, 1997; Lorence & Wagner, 1997), and *Alyxia* has its greatest species diversity in the Pacific (Middleton, 2000, 2002).

Chilocarpus and *Condylocarpon* are sister genera that are the sister group to the remainder of Alyxieae (Fig. 6). Because of its syncarpous ovary, *Chilocarpus* was included in Carisseae (Willughbeieae sensu Endress & Bruyns, 2000) by Schumann (1895, as Arduineae) and the invalid Chilocarpinae by Pichon (1948a). Leeuwenberg (1994a) provided a Latin diagnosis, raised Pichon's subtribe to tribal level, and in his recent revision of *Chilocarpus* (Leeuwenberg, 2002) considers Chilocarpeae to fall somewhere between Carisseae and Ambelanieae. *Condylocarpon* is the only New World member of Alyxieae and exhibits a set of deviating pollen features (tetrads, inaperturate, and reduced exine; Fig. 7) that strongly indicate the pollen to be paedomorphic (i.e., underdeveloped regarding pollen wall features, yet viable). The basically decussate tetrad configuration indicates *Condylocarpon* pollen to be derived from a 2-aperturate rather than from a 3-aperturate ancestor, providing additional support for its present position in Alyxieae (van der Ham et al., 2001). Despite its aberrant, inaperturate, nearly exineless pollen, *Condylocarpon* resembles *Chilocarpus* in a number of other morphological aspects, especially the distinctive globose head of the flower buds. The fruits exhibit some superficial similarities; in most species of both genera, they are moniliform and woody, although the gynoecium is syncarpous and dehiscent in *Chilocarpus* and apocarpous and indehiscent in *Condylocarpon* (Fallen, 1983b).

The second main cluster of Alyxieae in the combined analysis (Fig. 6) is the *Vinca* clade and includes *Cabucala*, *Petchia*, *Rauvolfia*, *Ochrosia*, *Neisosperma*, *Kopsia*, *Catharanthus*, and *Vinca*. All taxa in this clade are characterized by a differentiated style head with a distinct annulus at the base, but this is a plesiomorphic feature and is also found in other tribes (e.g., *Alstonia* R. Br., Alstonieae; *Allamanda*, Plumerieae). Similarly, the pollen morphology of this clade is unspecialized (Fig. 8). The occurrence of well-developed colpal and mesocolpial plates due to the presence of distinct supplementary endocolpi (absent in *Vinca*, weak in *Kopsia* and several taxa outside the Vinceae: *Acokanthera* G. Don, *Allamanda*, *Aspidosperma*, and *Plumeria*) is their most discriminating feature. Of the taxa in the *Vinca* clade, *Rauvolfia*, *Ochrosia*, and *Kopsia* were treated in Schumann's (1895) classification. He included *Rauvolfia* in Rauvolfinae, together with nine other genera, none of which shows a close relationship with

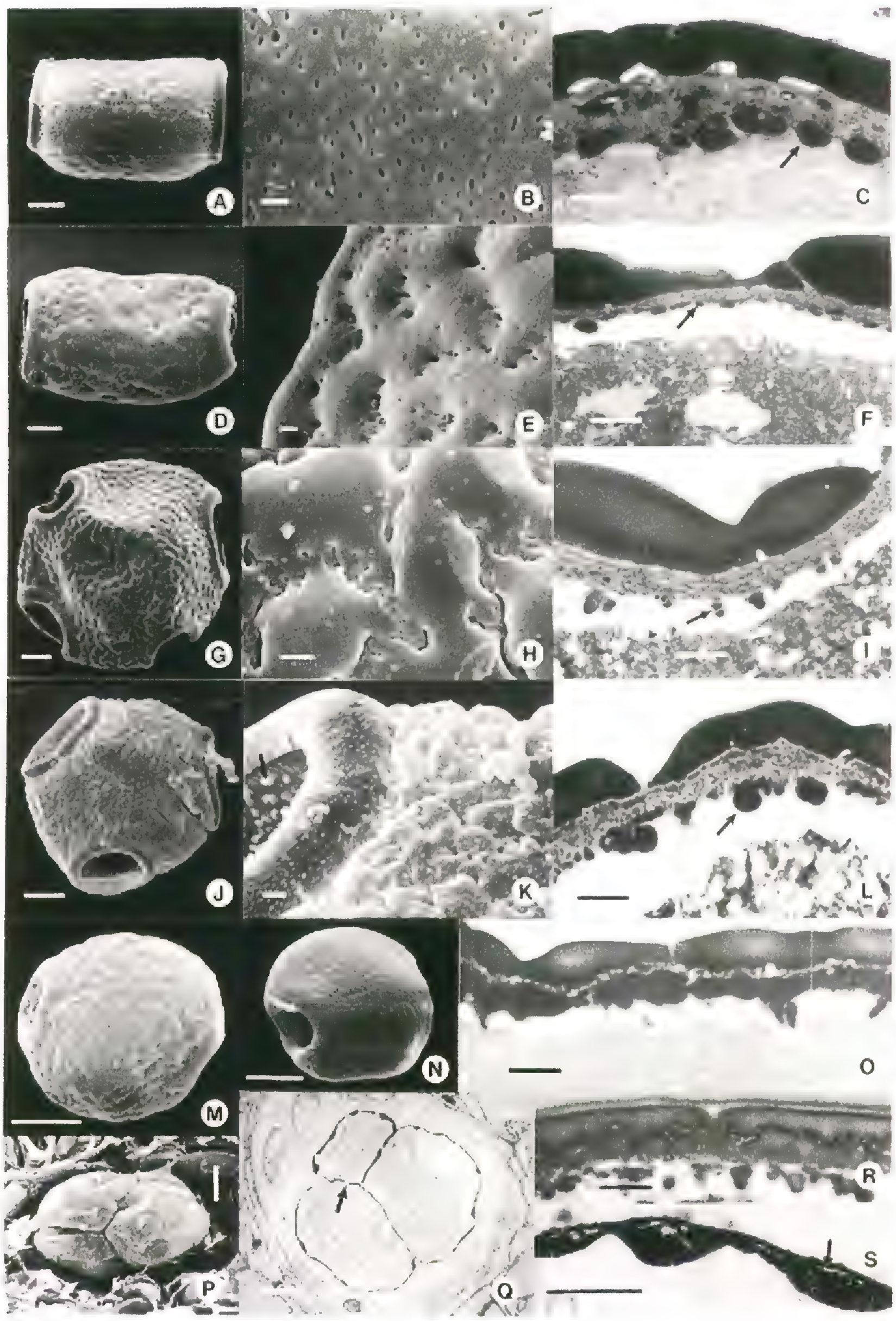

Figure 7. Apocynaceae, Alyxieae. SEM and TEM images of pollen grains. A–C. *Alyxia ruscifolia*. —A. Barrel-shaped 2-porate pollen grain (orientation unknown) with differently sized pores. —B. Detail of A, showing psilate, perforate ornamentation. —C. TEM section of exine and intine, showing tectum (above), infratectum (gaps), and endexinous inner layer with dark inclusions and dark granules on the inner surface (arrow). D, E. *Pteralyxia kauaiensis*. —D. Barrel-shaped 2-porate pollen grain (orientation unknown) with differently sized pores. —E. Detail of undulate ornamentation with perforations in the depressions. —F. *Pteralyxia macrocarpa*. TEM section of exine and intine, showing thick tectum (above), infratectum (commissural line and several small gaps), and endexinous inner layer with small dark inclusions and larger dark surface granules (arrow). G, H. *Ixpinia solomonensis*. —G. Tetraporate pollen grain (orientation unknown) with unequal sides and differently sized pores. —H. Detail of ± verrucate ornamentation. —I. *Ixpinia taitensis*. TEM section of exine and intine, showing thick tectum (above), infratectum (commissural line and gap), and endexinous inner layer with small dark inclusions and larger (locally stacked) dark surface granules (arrow). J–L. *Ixpiniopsis ternatensis*. —J. 3-porate pollen grain (orientation unknown) with unequal sides and differently sized pores. —K. Detail of J, showing ± verrucate ornamentation, psilate annulus, and inner surface granules (arrow) inside pore. —L. TEM section of exine and intine, showing thick tectum (above), infratectum (commissural line and sparse gaps), and endexinous inner layer with small dark inclusions and large dark surface granules (arrow). M, O. *Chilocarpus denudatus*. —M. Psilate to finely fossulate 2-porate pollen grain (orientation unknown). —O. TEM section of exine and intine, showing tectum (above), granular infratectum, and ectexinous inner verrucate layer. N, R. *Plectaneia thouarsii*. —N. Psilate, perforate 2-porate pollen grain (orientation unknown). —R. TEM section of exine and

Rauvolfia in this study or other recent analyses (Sennblad & Bremer, 2000, 2002; Potgieter & Albert, 2001; Simões et al., 2007), whereas *Ochrosia* and *Kopsia* were included in a different subtribe, Cerbereae (Plumerieae of Endress & Bruyns, 2000). Rauvolfieae as circumscribed by Pichon (1949a) included four subtribes. The Rauvolfinae comprised *Cabucala*, *Petchia*, and *Rauvolfia*, which are supported as monophyletic in the *Vinca* clade. Ochrosinae contained only *Ochrosia* (including *Neisosperma*), and Vallesinae included *Vallesia* and *Kopsia*, which are only distantly related here. His other two subtribes, Alyxinae and Condylocarpinae, belong to elements of *Alyxia* clade in our study. Pichon (1949a) included *Anechites* as a genus incertae sedis, noting that he did not have sufficient material to place it, but he thought that it most probably belonged with *Condylocarpon* or in a tribe of its own.

The classification of Leeuwenberg (1994a) followed Pichon's, with the same tribal circumscription of Alyxieae and the same subtribal circumscriptions. The only differences were that *Anechites* was included as a member of Condylocarpinae and the name of the tribe was changed to Alyxieae. More recently, Leeuwenberg (1997) placed *Cabucala* in synonymy under *Petchia*. Although there are some differences in pollen morphology (differing colpus length, tectum thickness and ornamentation, and the presence/absence of deviating mesocolpium centers) in the species studied, the floral structure of the two genera is nearly identical, and our results do not contradict the synonymy of *Cabucala*. *Neisosperma*, which was placed into synonymy under *Ochrosia* by Pichon (1949a) and maintained there by Leeuwenberg (1994a) and Hendrian (2004), is often considered to be a distinct genus, especially by specialists dealing with species of the Pacific Basin, where both genera have their greatest species density (Fosberg et al., 1977; Markgraf, 1979; Boiteau, 1981; Smith, 1988; Wagner et al., 1990; Forster & Williams, 1996). Macromorphologically, plants of *Neisosperma* and *Ochrosia* have a clear resemblance (e.g., trees with whorled leaves, corolla lobe aestivation dextrorsely contort, fruits large, fleshy colorful drupes). Their pollen, however, differs in several aspects (size, ectoaperture shape and margin, endoaperture margin, and ornamentation). In our analyses, inclusion of *Neisosperma* in *Ochrosia* is only moderately supported. A detailed study including more species of both genera is needed in order to elucidate their relationship.

Also in the *Vinca* clade (Fig. 6) are two genera conventionally included in Plumerieae: *Catharanthus* and *Vinca* (Schumann, 1895 as Alstoniinae; Pichon, 1949a as Alstonieae; Leeuwenberg, 1994a). The pollen of *Vinca* is peculiar and unique by its indistinct ectoapertures, relatively large endoapertures, and thin exine, which makes it difficult to compare with the pollen of other taxa. *Vinca* also shows some derived floral characteristics, such as the enlarged spathulate, apical anther appendage, which most likely plays a role in inhibiting desiccation of the secondarily presented pollen (Church, 1908), possibly related to the temperate habitat of this genus.

The genera included here in the *Vinca* clade are the same as those included in Vinceae of Endress and Bruyns (2000), with the exception of *Amsonia* and *Rhazya*. These two genera have traditionally been considered to be closely related to *Catharanthus* and *Vinca*, and thus conventionally included in Plumerieae (Schumann, 1895 as Alstoniinae). *Amsonia*, *Rhazya*, *Catharanthus*, and *Vinca* constituted Lochnerinae of Pichon (1949a) and Catharanthaninae of Leeuwenberg (1994a). Flowers, fruits, seeds, and pollen of *Rhazya* scarcely differ from those of *Amsonia* (Pichon, 1949a; Nilsson, 1986), which was therefore treated as a synonym of *Amsonia* by Endress and Bruyns (2000) and not contradicted by our study (Fig. 6). However, *Amsonia* and *Rhazya* together group here with neither Alyxieae nor Vinceae. Instead, they are placed as sister to the Plumerieae and Carisseae + Apocynoideae, although bootstrap support for this is less than 50% (Fig. 6). In the study by Potgieter and Albert (2001), *Amsonia* was in a clade together with *Thevetia peruviana*, which was included in a large polytomy. Floral structure of *Amsonia* and *Rhazya*, including details of the style head, is similar to that of *Catharanthus* and *Vinca* and does not agree with the more derived position for the former two genera indicated by the molecular analysis. Similarly, *Amsonia* and *Rhazya* occupy an unexpected position in the molecular tree considering their secondary

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intine, showing tectum (above), undulate granular infratectum, and ectexinous inner layer with surface verrucae (partly loose?). P, Q, S. *Condylocarpon isthmicum*. —P. Tetrad almost filling anther locule, showing the four constituent, psilate members in decussate configuration. —Q. TEM section through tetrad almost filling anther locule, showing three of the four constituent members, locally fused internal walls with sparse pores (arrow), and thin, locally thickened, external walls. —S. TEM section through external wall, showing the thin tectum, the poorly defined infratectum (arrow), and the locally thickened ectexinous inner layer. Scale bar = 10 μm in A, D, G, J, M, N, P, Q; scale bar = 1 μm in B, C, E, F, H, I, K, L, O, R, S.

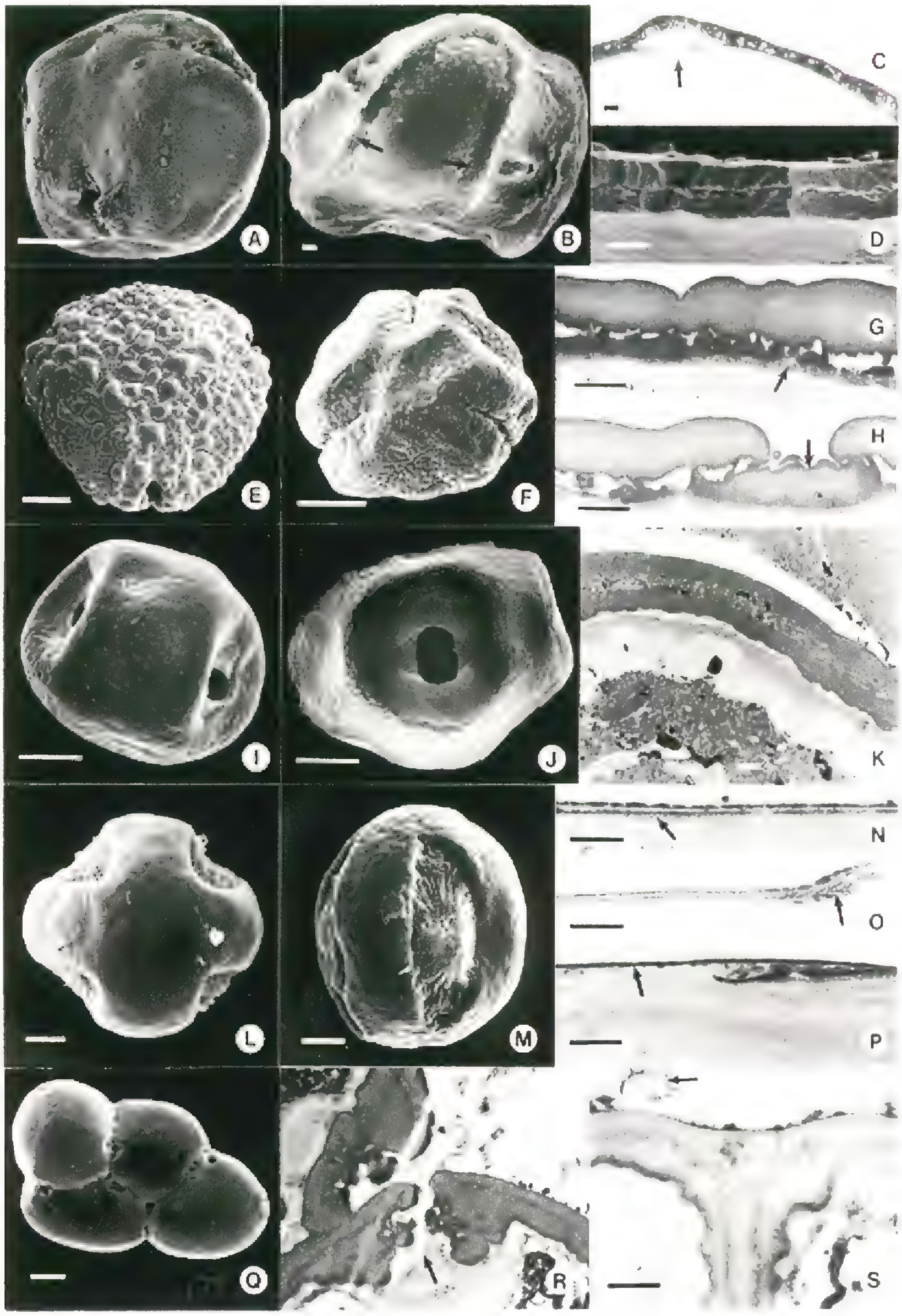

Figure 8. Apocynaceae, Vinceae (A–P), Melodineae (Q–S). SEM and TEM images of pollen grains. —A. *Petchia ceylanica*. Psilate 3-colporate pollen grain in oblique view; colpi short with relatively large endopore. B, C. *Cabucala caudata*. —B. Inside of tricolporate pollen grain showing two colpal plates, each enclosing an endopore surrounded by a distinct endoannulus; in the center a mesocolpial plate delimited from the colpal plates by supplementary endocolpi (arrows). —C. TEM section through supplementary endocolpus (arrow), colpal plate (left), and mesocolpial plate (right). —D. *Kopsia fruticosa*. SEM section, showing psilate to scabrate exine stratified into two equally thick strata (tectum above) separated by a thin infratectum. —E. *Ochrosia coccinea*. Verrucate 3-colporate pollen grain in polar view. F–H. *Neisosperma nakaiana*. —F. Psilate/finely fossulate tricolporate pollen grain in polar view. —G. TEM section through mesocolpial exine and intine, showing tectum (above), irregular infratectum and foot layer, and spongy endexine (arrow). —H. TEM section through apertural exine, showing tectum (above), irregular infratectum, foot layer, which is much thickened under the colpus (arrow), and spongy endexine. I–K. *Rhazya stricta*. —I. Psilate, perforate pollen grain in equatorial view; colpi short and wide with relatively large endopores. —J. Inside of pollen grain showing lalongate endopore delimited polewards by horizontally oriented colpal plates; to the left and to the right, psilate mesocolpial plates delimited by wide verrucate zones (supplementary endocolpi). —K. TEM section through exine and intine, showing tectum (above), slightly thinner, granular-reticulate infratectum, and inner layer of the same thickness as tectum. L, M. *Vinca minor*, (critical-point dried). —L. Psilate 1-aperturate pollen grain in polar view. —M. Psilate 1-aperturate pollen grain in equatorial view, showing the indistinct ectoaperture (porous area) in center of depressed oblong zone that is delimited by the endoapertural costae. N–P. *Vinca major*. —N. TEM section through thin mesocolpial exine and intine, showing tectum (above) with indistinct perforations, distinct

chemistry. Both genera contain numerous complex indole alkaloids of the plumerane type (Ganzinger & Hesse, 1976; Kisakürek et al., 1983), whereas in all genera above them in the uppermost clades of the tree and in Alyxieae, indole alkaloids have been lost. In the comparative study by Nilsson (1986), however, pollen of *Amsonia* and *Rhazya* was found to be nearly identical but showed no close relationships with pollen of *Catharanthus* or *Cabucala* (Vinceae). Because the focus of our study was Alyxieae, this was the only group densely sampled. To better assess the phylogenetic position of *Amsonia* and *Rhazya*, additional representatives of other tribes, especially previously unstudied genera of Melodineae sensu Endress and Bruyns (2000), should be included.

The unexpected positioning of *Amsonia* and *Rhazya* in the combined molecular tree (Fig. 5) away from the rest of Vinceae could be regarded as a case of incongruence with morphology. However, in the morphological analysis, *Amsonia* and *Rhazya* did not always appear together with *Catharanthus* + *Vinca*, and their position was relatively unclear (e.g., no BP > 50). Furthermore, combining the DNA and morphological data resulted in generally better resolution and higher support in the all-data combined tree relative to the combined DNA data tree, which would not be expected if there were highly incongruent basic patterns in each of them. The exact position of *Amsonia/Rhazya* is also not clear with the DNA data, except that their exclusion from the clade with the rest of Vinceae is not strongly supported (Figs. 5, 6), which concurs with the results of Simões et al. (2007).

SIGNIFICANCE OF MORPHOLOGICAL AND CHEMICAL CHARACTERS

As has been demonstrated here and elsewhere (Fallen, 1983a; Sennblad & Bremer, 2000, 2002; Potgieter & Albert, 2001), superficial resemblance of characters such as fruits and seeds of Apocynaceae that are correlated with dispersal mode are extremely labile, and there is a tendency for the repeated independent evolution of certain fruit and seed types. Similar findings for other angiosperm families have been reported by Bremer and Eriksson (1992), Armbruster (1996), Endress (1996), Hufford (1997), and Clausing et al. (2000). Fruit and seed characters are thus particularly unreliable when used alone for determining relationships among genera in Apocynaceae. Examples of these characters include: fruit dehiscence, mesocarp consistency, and seed appendages. It was the use of such simple, single character-based categories that led to the artificial tribal classifications of Schumann (1895), Pichon (1949a), and Leeuwenberg (1994a). The most reliable characters are likely to be more subtle, and one must be willing to invest some effort to determine what they are. It is also unrealistic to believe that any single character is going to provide a non-homoplasious synapomorphy for any large genus or tribe, but rather it is more reasonable to expect that these groups can be circumscribed by a specific combination of characters.

Alyxieae pollen is characterized by large porate apertures (Fig. 7), which are distinct from the usual small aperturate porate grains found in Apocynoideae, completely different from the colporate grains found in Vinceae, and characteristic for all other Rauvolfioideae (Figs. 8, 9). Details of the pollen ectoapertures proved to be the most important morphological characters for defining Alyxieae because the unusual and distinct pollen type is synapomorphic for the tribe. Aperture type is less informative in the other tribes of Rauvolfioideae because, with a few exceptions (e.g., *Craspidospermum*; Fig. 8), all Rauvolfioideae have colporate pollen grains. Within Alyxieae, aberrant, inaperturate, nearly exineless pollen that remains in tetrads is an autapomorphy for *Condylocarpon*. Inaperturate pollen is otherwise known in the family only in Secamonoideae and Asclepiadoideae as well as some genera of Periplocoideae that have pollinia (e.g., *Finlaysonia* Wall., *Hemidesmus* R. Br.; Schill & Jäkel, 1978; Verhoeven & Venter, 2001). *Condylocarpon* is also of interest biogeographically, being the only Neotropical member of Alyxieae; all other genera are found in southeastern Asia and the Pacific. Aperture type thus provides a clear distinction between the Alyxieae and Vinceae.

Other morphological characteristics that distinguish Alyxieae from Vinceae (but not necessarily

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infratectum, and inner layer consisting of foot layer and spongy endexine (arrow). —O. TEM section through endoapertural costa (arrow) in ectoapertural area. —P. TEM section through endoapertural costa outside ectoapertural area, showing continuous tectum (arrow), discontinuous inner exine, and slightly bulging intine. Q–S. *Craspidospermum verticillatum*. —Q. Rhomboidal tetrad, showing several pores (in adjacent positions) near sutures. —R. TEM section through two adjacent pores of neighboring grains, showing small ectopores and heavily costate endopore (arrow). —S. TEM section through two adjacent pollen grains, showing exine stratigraphy with joint tecta, separate infratecta, and inner exine layers; note Ubisch bodies (arrow). Scale bar = 10 μm in A, E, F, L, M, Q; scale bar = 5 μm in I–K; scale bar = 1 μm in B–D, G, H, N–P, R, S.

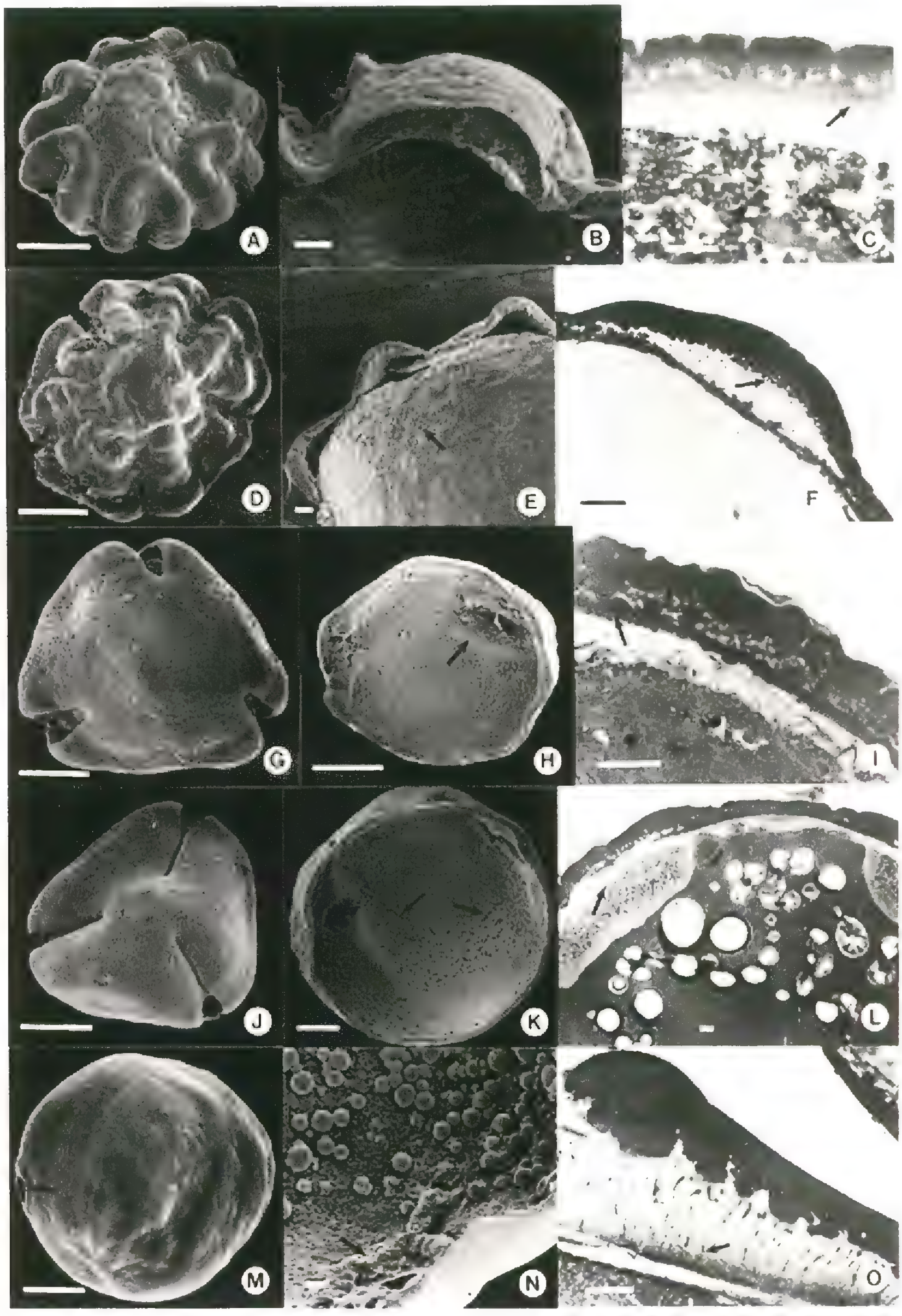

Figure 9. Apocynaceae. Aspidospermeae (A, B, D–F), Alstonieae (C), Hunterieae (G–I), Plumerieae (J), Carisseae (K, L). Malouetieae (Apocynoideae) (M–O). SEM and TEM images of pollen grains. A, B. *Aspidosperma parvifolium*. —A. Psilate/fossulate 6-colporate pollen grain in slightly oblique polar view, showing prominent arcus-like ridges surrounding the colpi. —B. SEM section of ridge, showing a thin, psilate, perforate tectum, thick granular infratectum, and thin inner exine layer. C. *Alstonia scholaris*. TEM section through exine and intine, showing thick psilate, perforate tectum, granular infratectum, dimerous foot layer, and indistinct spongy endexine (arrow). D–E. *Vallesia glabra*. D. Psilate pentacolporate pollen grain in polar view, showing prominent ridges surrounding colpi. —E. Part of fractured pollen grain showing three 'empty' ridges and psilate to scabrate inside with elongated, unevenly distributed granular marks (arrow). —F. *Vallesia antillana*. TEM section through ridge, showing tectum subtended by granular infratectum (arrow) and cavity, and a + dimerous inner layer. G, H. *Pleiocarpa mutica*. G. Psilate, perforate tricolporate pollen grain in polar view. —H. Scabrate inside of colporate pollen grain showing two apertures (right and extreme left); endopores have polar costae (arrow). —I. *Picralima nitida*. TEM section through exine and intine; exine consists of relatively thick, undulated tectum, slightly thinner granular-reticulate infratectum subtended by a thin foot layer and thin indistinct endexine (arrow). —J. *Anechites nerium*. Psilate, finely perforate tricolporate pollen grain in polar view. K, L. *Acokanthera oppositifolia*. —K. Inside of tricolporate pollen grain showing colpal plates with lalongate endopores (left and right) and system of endocracks (supplementary endocolpi; arrows) delimiting psilate mesocolpial and polar plates. —L. TEM section through supplementary endocolpus (arrow) subtended by thickened intine (left) and mesocolpial exine (right). M–O. *Mascarenhasia arborescens*. —M. Psilate triporate pollen grain, showing pores with weak annulus (arrow). —N. Exine fragment showing part of psilate, sparsely perforate tectum, inner side beset with diversely

from other tribes of Rauvolfioideae) include the simple style head, which is uniformly secretory and receptive and lacks a pollen-trapping annulus at the base (vs. the style head differentiated into distinct morphological and functional zones, with the receptive region located beneath an annulus at the base). Except for *Plectaneia*, Alyxieae seeds have a tough and usually conspicuously ruminate endosperm (vs. a smooth and soft endosperm in Vinceae).

Because tribes within Rauvolfioideae were so artificial in earlier classifications (Schumann, 1895; Pichon, 1949a, 1950b; Leeuwenberg, 1994a), it was impossible to understand the evolution of secondary chemistry in this subfamily. As tribal circumscriptions become more natural, it is now possible to gain a better understanding of the phylogenetic pattern of indole alkaloids and cardenolides. Generally, indole alkaloids are considered to be characteristic for Rauvolfioideae. However, indole alkaloids characterize only five of the tribes included in this study (Alstonieae, Tabernaemontaneae, Vinceae, Hunterieae, and Melodineae), whereas indole alkaloids have not been reported in the other three tribes (Fig. 6). *Acokanthera* and *Carissa* L. contain cardenolides. Based on their syncarpous gynoecium, they were conventionally included with indole alkaloid–containing genera in a polyphyletic Carisseae (Leeuwenberg, 1994a). Here and in other phylogenetic studies (Endress et al., 1996; Potgieter & Albert, 2001; Simões et al., 2007), the Carisseae sensu Endress and Bruyns (2000) are placed with other taxa in which indole alkaloids have been lost or replaced by other secondary compounds (Johns et al., 1968; Hegnauer, 1970; Coppen & Cobb, 1983; Kisakürek et al., 1983; Jensen, 1992).

All genera of Vinceae contain various complex indole alkaloids (Hegnauer, 1970, 1989; Ganzinger & Hesse, 1976; Kisakürek et al., 1983). In contrast, all genera of Alyxieae studied for secondary chemistry lack indole alkaloids. *Alyxia* and *Lepiniopsis* contain coumarins (Johns et al., 1968; Hegnauer, 1970, 1989), and although *Lepinia* has never been analyzed for secondary compounds, the crushed leaves are known to emit a strong coumarin scent (D. Lorence, pers. comm., 1999). Coumarins are absent from *Plectaneia*, and no reliable data are available on the secondary chemistry of *Pteralyxia*, *Chilocarpus*, and *Condylocarpon*.

CHARACTER EVOLUTION

Gynoecium, fruit, and seed morphology are complex and homoplasious in Rauvolfioideae. In this analysis, the plesiomorphic state of the gynoecium is apocarpous, in concurrence with Potgieter and Albert (2001; but see Sennblad & Bremer, 2000, 2002, for an alternative view), and the majority of the genera are apocarpous. Even in the small sample here, almost every clade includes at least one syncarpous genus; similar findings are reported by Simões et al. (2007).

The style head is a useful character for distinguishing genera in Apocynaceae (see Fig. 10), but it is structurally complex, making it difficult to break down into meaningful character states for coding morphological characters. The two taxa sister to the rest in this analysis, *Vallesia* and *Aspidosperma*, have a simple style head that is vertically undifferentiated. A simple style head is also characteristic for all Alyxieae and for the Hunterieae and Carisseae (Fallen, 1986; Endress et al., 1996; Endress & Bruyns, 2000). A similar type of style head is found in some (but not all) genera of Alstonieae and Plumerieae. *Tabernaemontana* s.l., as currently circumscribed by Leeuwenberg (1991, 1994b), includes species with a simple, undifferentiated style head, such as the species included in this study, as well as ones with a complex, vertically differentiated style head with distinct functional regions and a broad pollen-trapping flange at the base (e.g., all the New World species). These results indicate that style head specialization has probably evolved in parallel in various clades of Rauvolfioideae, as was suggested by Potgieter and Albert (2001), and that this probably has proceeded in both directions (Fig. 10).

Within Alyxieae, *Lepinia* and *Lepiniopsis* have a 3- to 5-carpellate ovary, which is partially to completely syncarpous, respectively (Endress et al., 1997). A 3- to 5-carpellate ovary is otherwise known in Apocynaceae only in *Pleiocarpa* (Hunterieae). All other genera have two carpels.

Fruit type and seed margin are equivocally optimized: *Vallesia* has naked seeds in a small juicy drupe, whereas *Aspidosperma* has thick woody follicles and seeds with a diaphanous wing. This is in sharp contrast to the traditional view of the berries of Carisseae (a conglomeration of Willughbeieae, Melodineae, Hunterieae, and Carisseae, sensu Endress &

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sized granules, and endoannulus (arrow). —O. TEM section through exine and intine near pore; exine is thickened into an ecto- and endoannulus; innermost exine layer consists of granules or irregular elements that are separated from tectum by granular infratectum; the intine contains numerous radially oriented dark inclusions (arrow). Scale bar = 10 μm in A, D, G–J; scale bar = 5 μm in K, M; scale bar = 1 μm in B, C, E, F, L, N, O.

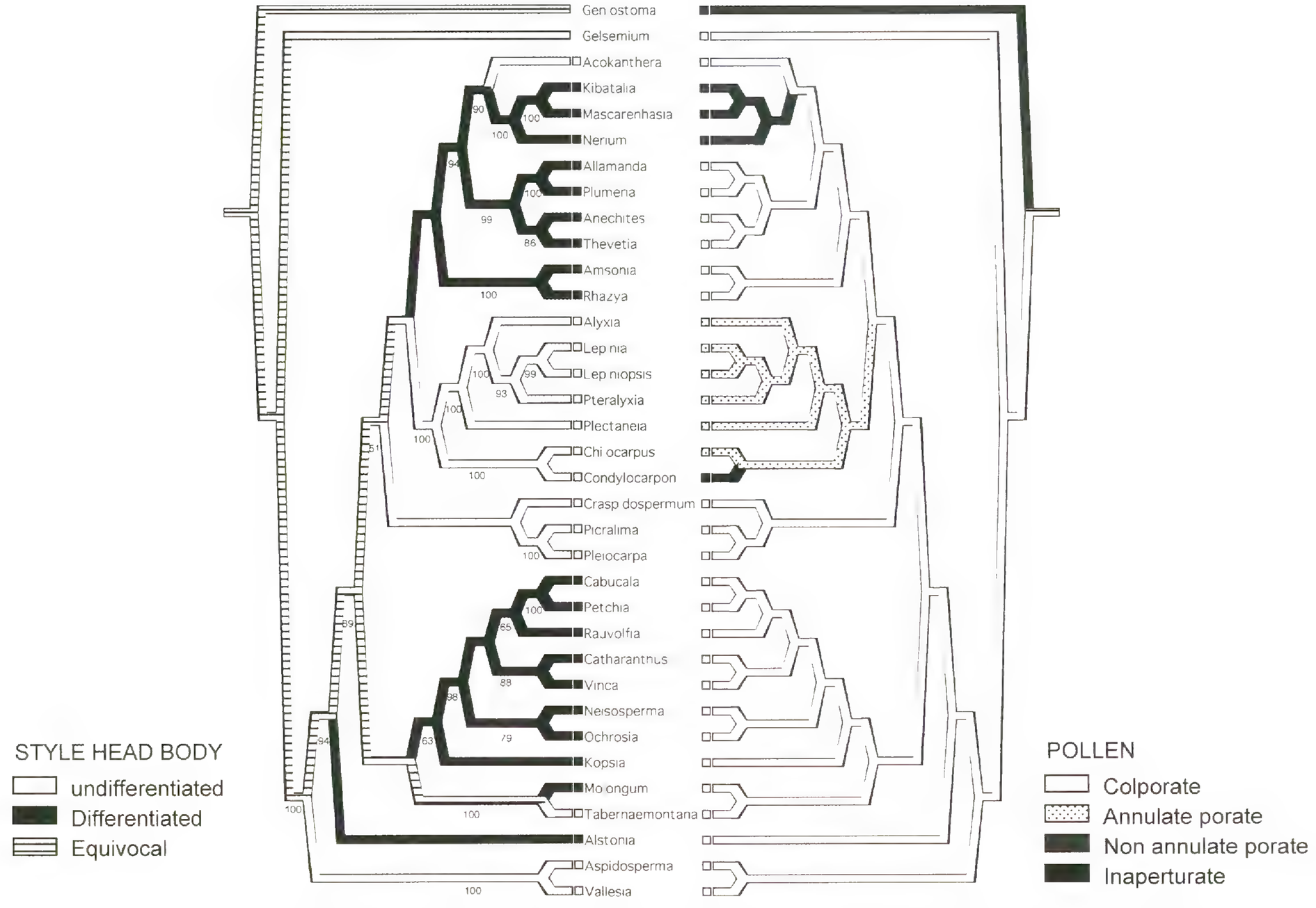
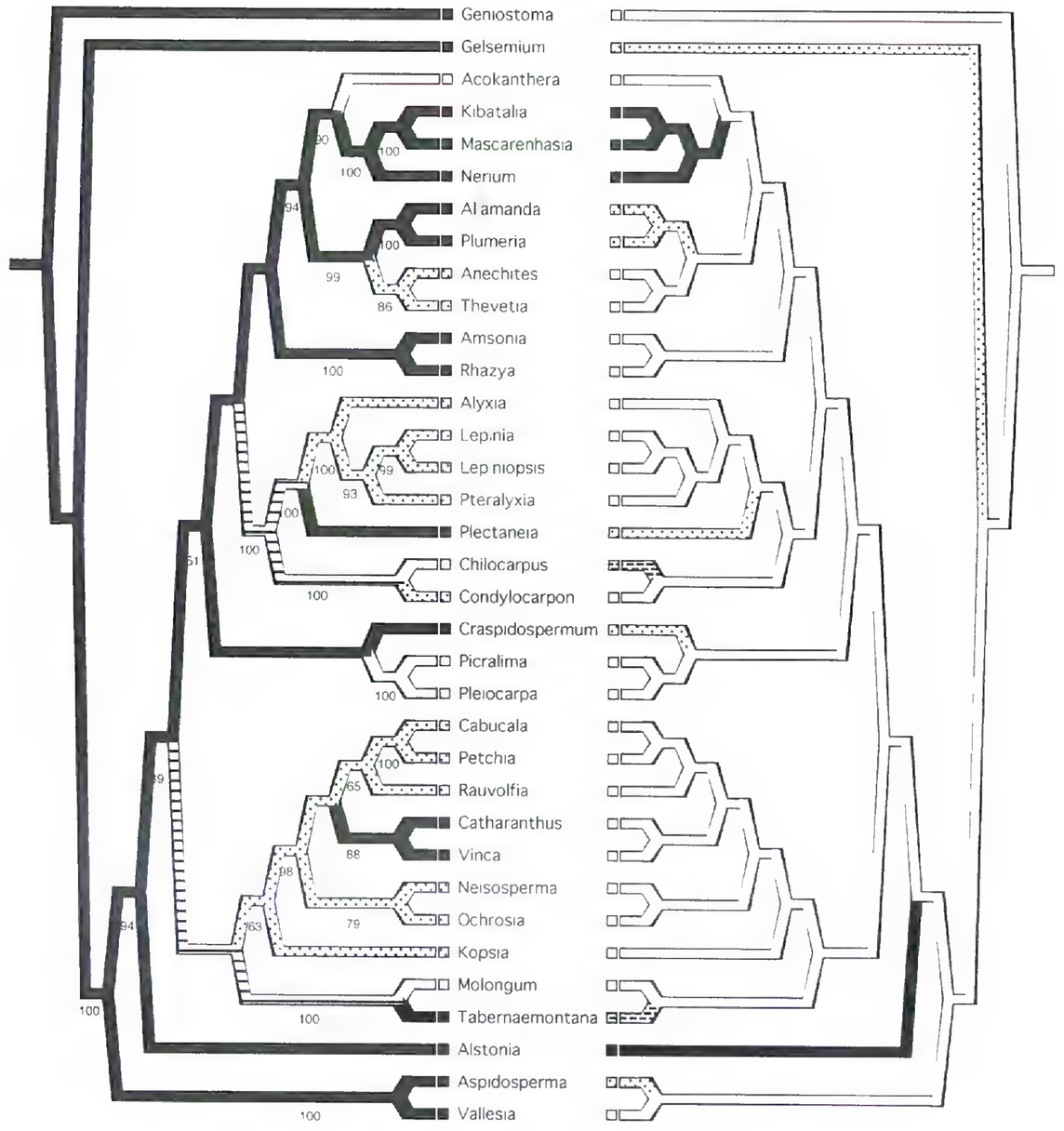


Figure 10. Evolution of style head body differentiation and pollen aperture type mapped onto the single most parsimonious tree from the combined analysis of morphological and molecular data using ACCTRAN optimization.



FRUIT TYPE

- Berry
- Drupe
- Dry dehiscent follicle or capsule
- Fleshy dehiscent follicle or capsule
- Equivocal

SEED MARGIN

- Naked
- Winged
- Arillate
- Comose
- Fimbriate

Figure 11. Evolution of fruit type and seed margin mapped onto the single most parsimonious tree from the combined analysis of morphological and molecular data using ACCTRAN optimization.

Bruyns, 2000) as the most unspecialized fruit type in the family. Vinceae are homogeneous with regard to seed margin, with the seeds being unwinged (see Simões et al., 2007, regarding *Kamettia* and *Tonduzia*, not included here). In the genera included in our analysis, the seeds are enclosed in an indehiscent drupe. Only *Catharanthus* and *Vinca* deviate, in having delicate, papery dehiscent follicles (Fig. 11). *Catharanthus* and *Vinca* also have a derived herbaceous habit, whereas the other genera in Vinceae are trees or shrubs.

The seed margin is evolutionarily plastic in Alyxieae (Fig. 11). The four morphologically most specialized genera—*Alyxia*, *Lepinia*, *Lepiniopsis*, and *Pteralyxia*, as well as *Condylocarpon*—all have distinctive cylindrical seeds that are longitudinally rolled with a deep hilar groove. *Plectaneia*, in contrast, has flat seeds with a wing at each end. In *Chilocarpus* the seeds have a small pink corky aril on the funiculus and are presented in an unusual type of leathery dehiscent fruit that splits apart along one or two valves (Leeuwenberg, 2002). Arils are rare in Apocynaceae, otherwise known only in Tabernaemontaneae. Similar types of fruits are known as display fruits in Gesneriaceae (Wiehler, 1983; Smith, 2000) and Melastomataceae (Clausing et al., 2000) or as dehiscent berries in Oleaceae (Lawrence & Green, 1993; Li et al., 2002). Seed margins also vary considerably in Plumerieae (Pichon, 1949b, 1950b as Cerberoideae). In contrast to this diversity in Rauvolfioideae, in Apocynoideae and all other subfamilies, the fruit and seed type is uniform: the fruit is a pair of follicles (rarely postgenitally united) that dehisce to release small, comose seeds.

Palynologically, starting from the basic regular 3-colporate condition typical in the Rauvolfioideae (Figs. 8, 9; Nilsson, 1986), an entire suite of changes characterizes the derivation of Alyxieae (Fig. 10): pollen grain shape irregular, aperture number mostly two (sometimes three), and ectoapertures porate with thickened margins (Fig. 7). Within the clade, several other characters change: maximum pore diameter is 9 μm in *Chilocarpus*, 12 μm in *Plectaneia*, and 21–33 μm in the subclade including *Alyxia*, *Pteralyxia*, *Lepinia*, and *Lepiniopsis*. Possibly due to paedomorphosis, *Condylocarpon* pollen is inaperturate (van der Ham et al., 2001). Together with the larger maximum pore diameter, the *Alyxia* clade also shows much larger, barrel-shaped (two pores) or depressed (three or four pores) pollen grains, an endexinous inner exine layer with a granular surface, and an indistinct (reduced) infratectum. Within the *Alyxia* clade, there is a change toward a more heavily sculptured tectum in the subclade including *Alyxia*, *Pteralyxia*, *Lepinia*, and *Lepiniopsis*, from psilate in *Alyxia*, via undulate in *Pteralyxia*, toward ± verrucate in *Lepinia* and *Lepiniopsis*. Pollen of the last two genera mostly has more than two apertures: three (less often two) in *Lepiniopsis* and three or four in *Lepinia* (van der Ham et al., 2001). This is not simply a reversal to basic aperture conditions, as the apertures are still irregular or diverse within a single grain regarding size, configuration, and orientation.

The functional significance of the remarkable shift in pollen morphology toward and within Alyxieae is not understood. The oldest fossils of the *Alyxia* pollen type date from the Paleocene of northwestern Borneo (Muller, 1981), which demonstrates the considerable age of the syndrome.

CLASSIFICATION

The topologies of this study support recognition of Vinceae and Alyxieae sensu Endress and Bruyns (2000), as well as the exclusion of *Vallesia* and *Anechites* from either tribe and their placement in Aspidospermeae sensu Simões et al. (2007) and Plumerieae, respectively. Our results do not support inclusion of *Amsonia* and *Rhazya* in Vinceae; however, these two genera are not supported in any of the other groups included in this study. The position of *Amsonia* was also not resolved in the study by Simões et al. (2007). Because the position of *Amsonia* and *Rhazya* remains equivocal, these genera are withdrawn from Vinceae and left unplaced for the time being. Although it is unsatisfying to leave them in limbo, a classification should reflect phylogeny, so it seems best to keep them as unplaced genera until more data are available to place them more definitely.

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Appendix 1.

Voucher specimens used for morphological character assessment in the Alyxieae study. Herbarium acronyms are in parentheses. Specimens used to study floral structure are indicated with FS, those used for light microscopy, scanning electron miscroscopy, and transmission electron microscopy of pollen grains are indicated by LM, SEM, and TEM, respectively, following the herbarium acronym.

APOCYNACEAE

Acokanthera oblongifolia (Hochst.) Codd
cult. Bot. Gard. Wageningen, *Kas et al. s.n.* (Z) FS

Acokanthera oppositifolia (Lam.) Codd
South Africa, *Bayliss BRI 544* (S) LM, SEM, TEM

Allamanda cathartica L.
cult. Royal Bot. Gard. Kew, 1983, *Fallen s.n.* (Z) FS; Gabon, *Leeuwenberg 12540* (WAG) LM, SEM, TEM

Alstonia scholaris (L.) R. Br.
cult. Fairchild Trop. Gard., *Gillis 6995* (Z) FS; New Guinea, *Schodde 2472* (L) LM, SEM, TEM

Alyxia oblongata Domin
Australia, *Dockrill 835* (L) LM

Alyxia ruscifolia R. Br.
cult. Montpellier, *Civeyrel 1055* (Z) FS; Australia, *Clark et al. 1753* (L) SEM, TEM

Amsonia ciliata Walter
U.S.A., *Sasseen s.n.* (WAG) LM, SEM, TEM

Amsonia tabernaemontana Walter
cult. Bot. Gard. Zürich, *Endress s.n.* (Z) FS

Anechites nerium (Aubl.) Urb.
Ecuador, *Asplund 16471* (Z) FS; Dominican Republic, *Ekman 15239* (S) LM, SEM

Aspidosperma parvifolium A. DC.
Brazil, Ferreira s.n. (Z) FS; Brazil, Heringer 10672 (UB) LM, SEM

Cabucala caudata Markgr.
Madagascar, *Capuron 23701-SF* (P) LM, SEM, TEM

Cabucala polysperma (Scott-Elliot) Pichon
Madagascar, *Civeyrel 1281* (Z) FS

Catharanthus roseus (L.) G. Don
cult. Bot. Gard. Zurich, *Endress s.n.* (Z) FS; Liberia, *Van Harten 29* (WAG) LM, SEM, TEM

Chilocarpus denudatus Blume
cult. Bot. Gard. Bogor, *Burck s.n.* (Z) FS; India, *Ridsdale 757* (L) LM; Java, *Blume s.n.* (L) LM; Java, *anon. s.n.* (S) SEM; Sarawak, *Richards 1463* (L) TEM

Chilocarpus suaveolens Blume
Java, Hochreutiner 2547 (L, Z) FS

Condylocarpon guyanense Desf.
French Guiana, *Sastre 5470* (P, Z) FS

Condylocarpon isthmicum (Vell.) A. DC.
Brazil, *Koch s.n.* (Z) FS; Brazil, *Hatschbach 13030* (Z) LM, SEM, TEM

Craspidospermum verticillatum Bojer ex A. DC.
Madagascar, *Civeyrel 1234* (Z) FS, LM, SEM, TEM

Kibatalia gitingensis (Elmer) Woodson
Philippines, *Liede 3268* (Z) FS; Philippines, *Wenzel 652* (G) LM, SEM, TEM

Kopsia fruticosa (Ker Gawl.) A. DC.
Java, *Prévost 167* (Z); cult. Victoria, Trinidad, *Broadway 5963* (S) LM, SEM, TEM

Lepinia marquisensis Lorence & W. L. Wagner
Fatu Hiva, Marquesas Islands, *Perlman 10271* (BISH, Z) FS

Lepinia solomonensis Hemsl.
Solomon Islands, *BSIP 13496* (L) LM, TEM

Lepinia taitensis Decne.
Society Islands, Moorea, *Perlman et al. 15071* (PTBG, Z) FS; Society Islands, Tahiti, *Whistler 4932* (BISH) SEM

Lepiniopsis ternatensis Valeton
Moluccas, *Mochtar 306* (L) LM, SEM; *PNH 17362* (L) TEM

Lepiniopsis trilocularis Markgr.
Palau Islands, *Lorence 8265* (PTBG, Z) FS

Mascarenhasia arborescens A. DC.
cult. Fairchild Bot. Gard., *Bird s.n.* (Z) FS; Madagascar, *Schlieben 8128* (Z) LM, SEM; Madagascar, *Capuron 22808-SF* (P) LM, SEM, TEM

Molongum laxum (Benth.) Pichon
Venezuela, *Berry 5400* (MO, Z) FS; Colombia, *Duntii 36267* (COL) LM, SEM, TEM

Neisosperma nakaiana (Koidz.) Fosberg & Sachet
cult. Waimea Arboretum, Hawaiian Is., *Neill 5291* (Z) FS, LM, SEM, TEM

Nerium oleander L.
cult. Bot. Gard. Zurich, *Fallen s.n.* (Z) FS; France, *Segal 232* (WAG) LM, TEM; cult. Perpignan, *Leeuwenberg 12206* (WAG) LM, SEM, TEM

Ochrosia coccinea (Teijsm. & Binn.) Miq.
cult. Bogor Bot. Gard., Java, *anon. s.n. 30/8/1982* (Z) FS, LM, SEM, TEM

Petchia ceylanica (Wight) Livera
cult. Bot. Gard. Kaiserslautern, *Omlor s.n., Kessler s.n.* (Z) FS; Sri Lanka, *Wambeck 2510* (S) LM, SEM

Picralima nitida (Stapf) T. Durand & H. Durand
cult. Bot. Gard. Wageningen, *Leeuwenberg 10779* (Z) FS; Zaire, *Gille 100* (BR) LM, SEM, TEM

Plectaneia stenophylla Jum.
cult. Madagascar, Petignat s.n. (Z) FS

Plectaneia thouarsii Roem. & Schult.
Madagascar, *Bernardi 11820* (L) LM, SEM, TEM

Pleiocarpa mutica Benth.
cult. Bot. Gard. Wageningen, *van Setten 415* (WAG, Z) FS; Ivory Coast, *Leeuwenberg 12145* (WAG) LM, SEM

Plumeria rubra L.
cult. Bot. Gard. Zurich, *Fallen s.n.* (Z) FS; Ghana, *Leeuwenberg 11089* (WAG) LM, SEM, TEM

Pteralyxia kauaiensis Caum
Kauai, *Perlman 15456* (Z) FS; Hawaii, *Flynn 269* (PTBG) SEM

Pteralyxia macrocarpa (Hillebr.) K. Schum.
Hawaii, *Swezey s.n.* (L) LM, TEM

Rauvolfia vomitoria Afzel.
Ivory Coast, *Aké Assi s.n.* (Z) FS; Nigeria, *Leeuwenberg 11337* (WAG) LM, SEM, TEM; Ivory Coast, *Leeuwenberg 12122* (WAG) LM, SEM

Rhazya stricta Decne.
Yemen, *Brunner 31* (Z) FS; Saudi Arabia, *Schimper 812* (L) LM, SEM, TEM
Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult.
cult. Bot. Gard. Calcutta, *anon. s.n.* (Z) FS
Tabernaemontana pandacaqui Lam.
Australia, *Alkin s.n.* (Z) LM, SEM, TEM
Thevetia peruviana (Pers.) K. Schum.
cult. Bot. Gard. Zurich, *Fallen s.n.*, (Z) FS; cult. Florida, *Gillis 9227* (S) LM, SEM, TEM
Vallesia antillana Woodson
cult. Fairchild Trop. Gard, *Zona s.n.* (Z) FS; Florida, *Killip 43415* (S) TEM
Vallesia glabra (Cav.) Link
Galapagos Is., *A. & H. Andersen 1009* (QCA) LM, SEM
Vinca minor L.
cult. Zurich, *Fallen s.n.* (Z) FS; cult. Schipluiden, Netherlands, *De Kort s.n.* (L) SEM; cult. Sollentuna, Sweden, *Nilsson s.n.* (S) SEM
Vinca major L.
cult. Pijnacker, Netherlands, *Van der Ham s.n.* (L) LM, TEM

GELSEMIACEAE

Gelsemium sempervirens (L.) J. St.-Hil.
U.S.A., Louisiana, *Tucker 28771* (Z) FS; South Carolina, *Wall. s.n.* (S) LM, SEM, TEM

LOGANIACEAE

Geniostoma rupestre (J. R. Forst. & G. Forst.) var. *ligustrifolium* (A. Cunn.) B. J. Conn
New Zealand, *Garnock-Jones s.n.* (WELTU, Z) FS; New Zealand, *Nilsson NZ 9* (S) LM, SEM, TEM

Appendix 2. Voucher specimens used for molecular analyses and GenBank accession numbers.

Taxon		GenBank Accession No.			
APOCYNACEAE	Voucher/Literature Citation	*mat*K	*rbc*L	*trn*L	*trn*L-F
Acokanthera oblongifolia (Hochst.) Codd	Endress et al., 1996	Z70182			
	Sennblad & Bremer, 1996		X91758		
Acokanthera oppositifolia (Lam.) Codd	Potgieter & Albert, 2001			AF214302	AF214148
Allamanda cathartica L.	Endress et al., 1996	Z70190			
	Sennblad & Bremer, 1996		X91759		
Allamanda indet.	Potgieter & Albert, 2001			AF214304	AF214150
Alstonia boonei De Wild.	Potgieter & Albert, 2001			AF102374	AF214151
Alstonia scholaris (L.) R. Br.	Endress et al., 1996	Z70189			
	Sennblad & Bremer, 1996		X91760		
Alyxia buxifolia R. Br.	Potgieter & Albert, 2001			AF214306	AF214152
Alyxia ruscifolia R. Br.	cult. Montpellier, *Civeyrel 1055* (TL)	DQ837536			
	Sennblad & Bremer, 2002		AJ419731		
Amsonia tabernaemontana Walter	Potgieter & Albert, 2001			AF214307	AF214153
	cult. Royal Bot. Gard. Kew, *Civeyrel 1057* (TL)	AM295066	AM295078		
Anechites nerium (Aubl.) Urb.	Sennblad & Bremer, 2002		AJ419733	AM295087	AM295087
Aspidosperma quebracho-blanco Schltdl.	Potgieter & Albert, 2001			AF214319	AF214165
Aspidosperma triternatum Rojas Acosta	cult. Bot. Garden, Meise, *Bremer 3029* (UPS)	AM295077			
	Sennblad & Bremer, 2002		AJ419735		
Cabucala polysperma (Scott-Elliott) Pichon	Madagascar, *Civeyrel 1281* (TL)	AM295067	AM295079	AM295088	AM295088
Catharanthus roseus (L.) G. Don	Potgieter & Albert, 2001			AF102392	AF214175
	cult. Stockholm Univ., *Bremer 3128* (UPS)	AM295068			
	Sennblad & Bremer, 1996		X91757		
Chilocarpus suaveolens Blume	Endress et al., 1996	Z70184	X92445		
	cult. Bot. Gard. Bogor, *Chase 1208*			AM295089	AM295089
Condylocarpon amazonicum (Markgr.) Ducke	Potgieter & Albert, 2001			AF214337	AF214183
Condylocarpon guyanense Desf.	French Guiana, *M. F. Prevost s.n.* (CAY)	DQ837537	AM295080		
Craspidospermum verticillatum Bojer ex A. DC.	Madagascar, *Civeyrel 1234* (TL)	DQ837538		AM295090	AM295090
	Sennblad & Bremer, 2002		AJ419743		
Kibatalia gitingensis (Elmer) Woodson	Philippines, *Liede 3268* (Z)	AM295069			
	Sennblad & Bremer, 2002		AJ419745		
Kopsia fruticosa (Ker Gawl.) A. DC.	cult. Bot. Gard. Meise, *Bremer 3033* (UPS)			AM295091	AM295091
	Endress et al., 1996	Z70178			
	Sennblad & Bremer, 1996		X91763		
Lepinia taitensis Decne.	Sennblad & Bremer, 2002		AJ419746		
Lepiniopsis ternatensis Valeton	Potgieter & Albert, 2001			AF214374	AF214220
Lepiniopsis trilocularis Markgr.	Palau Islands, *Lorence 8265* (PTBG)		AM295081		

Appendix 2. Continued.

Taxon		GenBank Accession No.			
APOCYNACEAE	Voucher/Literature Citation	*mat*K	*rbc*L	*trn*L	*trn*L-F
Mascarenhasia arborescens A. DC.	Potgieter & Albert, 2001			AF214378	AF214224
	cult. Wageningen Agric. Univ., nr. 80-16, *Setten 625* (WAG)	AM295070			
	Sennblad et al., 1998		AJ002885		
Molongum laxum (Benth.) Pichon	Potgieter & Albert, 2001			AF214383	AF214229
	Endress et al., 1996	Z70185	X91765		
Neisosperma nakaiana (Koidz.) Fosberg & Sachet	Potgieter & Albert, 2001			AF214385	AF214231
Neisosperma oppositifolia (Lam.) Fosberg & Sachet	cult. Nat. Trop. Bot. Gard., Kauai, *Lorence s.n.*, NTBG 970511 (PTBG)	AM295071	AM295082		
Nerium oleander L.	Potgieter & Albert, 2001			AF214386	AF214232
	Civeyrel et al., 1998	Z98173			
	Sennblad et al., 1998		AJ002886		
Ochrosia coccinea (Teijsm. & Binn.) Miq.	Sennblad & Bremer, 2002		AJ419752		
	cult. Wageningen Agric. Univ. nr. 78PTHB 354, *van der Laan 389* (WAG)			AM295092	AM295092
Petchia ceylanica (Wight) Livera	cult. Univ. Kaiserslautern, Germany, *Omlor s.n.* (Z)	AM295072	AM295083	AM295093	AM295093
Picralima nitida (Stapf) T. Durand & H. Durand	Potgieter & Albert, 2001			AF214404	AF214250
	Endress et al. 1996	Z70179			
	Sennblad & Bremer, 1996		X91766		
Plectaneia stenophylla Jum.	Madagascar, *Petignat s.n.* (Z)		AM295084		
	Potgieter & Albert, 2001			AF214405	AF214251
Pleiocarpa mutica Benth.	Potgieter & Albert, 2001			AF214407	AF214253
	cult. Royal Bot. Gard. Kew, *Civeyrel 1086* (TL)	DQ837539			
	Sennblad & Bremer, 2002		AJ419757		
Plumeria alba Kunth	Potgieter & Albert, 2001			AF214408	AF214254
Plumeria inodora Jacq.	Sennblad & Bremer, 1996		X91767		
Plumeria rubra L.	Endress et al., 1996	Z70191			
Pteralyxia kauaiensis Caum	Kauai, Hawaii, *Lorence 7768* (PTBG, Z)	AM295073	AM295085	AM295094	AM295094
Rauvolfia mannii Stapf	Endress et al., 1996	Z70181			
	Sennblad & Bremer, 1996		X91769		
Rauvolfia serpentina Benth. ex Kurz	Potgieter & Albert, 2001			AF214415	AF214261
Rhazya stricta Decne.	*Agosti 29* (Z)	AM295074	AM295086	AM295095	AM295095
Tabernaemontana citrifolia L.	Potgieter & Albert, 2001			AF214431	AF214277
Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult.	Endress et al., 1996	Z70187			
	Sennblad & Bremer, 1996		X91772		
Thevetia ahouai (L.) A. DC.	Potgieter & Albert, 2001			AF214435	AF214281
Thevetia peruviana (Pers.) K. Schum.	Endress et al., 1996	Z70188			
	Sennblad & Bremer, 1996		X91773		
Vallesia antillana Woodson	cult. Fairchild Trop. Gard., *Meagher 966* (FTG)	AM295075			
	Sennblad & Bremer, 2002		AJ419767		
	Potgieter & Albert, 2001			AF214447	AF214293

Appendix 2. Continued.

Taxon		GenBank Accession No.			
APOCYNACEAE	Voucher/Literature Citation	*mat*K	*rbc*L	*trn*L	*trn*L-F
Vinca minor L.	cult. Bot. Gard. Uppsala, *Sennblad 230* (UPS)	AM295076			
	Sennblad & Bremer, 2002		AJ419768		
	Potgieter & Albert, 2001			AF214449	AF214295
GELSEMIACEAE					
Gelsemium sempervirens (L.) J. St.-Hil.	Endress et al., 1996	Z70195			
	Olmstead et al., 1993		L14397		
	cult. Royal Bot. Gard. Kew, *Civeyrel 1069* (TL)			AM295096	AM295096
LOGANIACEAE					
Geniostoma rupestre J. R. Forst. & G. Forst.	Endress et al., 1996	Z70194	Z68828		
	Wellington, New Zealand, *Garnock-Jones 2200* (WELTU)			AM295097	AM295097

APPENDIX 3. Matrix based on the morphological character coding. For some genera more than one species was used for character coding. a = species used to code characters 1–37 (the non-pollen characters); b = species used to code characters 38–54 (the pollen characters). See Appendices 1 and 4.

	1	2	3	4	5
	12345678901234567890123456789012345678901234
Acokanthera oblongifolia[a], *A. oppositifolia*[b]	01000000000000001000000000000201101000020100110011001
Allamanda cathartica	11200001100110012111101201020020001000102000010010120 0
Alstonia scholaris	01200010000000002011001200030020212000002000201000110 0
Alyxia oblongata[b], *A. ruscifolia*[a,b]	01201000000000001000001010100202010000111221001032200 0
Amsonia ciliata[b], *A. tabernaemontana*[a]	21100001000000002110001200100020213000002000021001120 0
Anechites nerium	11000101100000112011101201000020 21???0?020000001?1240
Aspidosperma parvifolium	01100010000000001001001201020020103000003030010007?1201
Cabucala caudata[b], *C. polysperma*[a]	01200000000000002110001010000000112000002000120001110 1
Catharanthus roseus	21000000000000002110201200100120213000102000220000120 1
Chilocarpus denudatus[a,b], *C. suaveolens*[a]	11001000001000001001001201300110 00???0011220101021110 0
Condylocarpon guyanense[a], *C. isthmicum*[a,b]	11200000001000001000001210100212 01???1010????0?1011200
Craspidospermum verticillatum	01200010000000002011000200010020213001013300100001110 0
Kibatalia gitingensis	01000201010111023000101201201310110010002310100031120 0
Kopsia fruticosa	01000000010000002110201110000 01--13000102000210001110 1
Lepinia marquisensis[a], *L. solomonensis*[b], *L. taitensis*[a,b]	01100000001000001000011110100202010000112221001032201 0
Lepiniopsis ternatensis[b], *L. trilocularis*[a]	01100000001000001000011110100202010000112221001032201 0
Mascarenhasia arborescens	01000101010011023000101201201310110010002310101031100 0
Molongum laxum	01000100000111012131001000300030 11???0003120101021110 0
Neisosperma nakaiana	01200000010000002120001110010010112000002000220000100 1
Nerium oleander	01200111010001023121001201201310110100012310 1?0311000
Ochrosia coccinea	01200000010000002120001010010010112000102120020000102 1
Petchia ceylanica	01200000000000002110001010000000 11???0002100120021100 0
Picralima nitida	01000110000000001000001100300030012000002100200010104 0
Plectaneia stenophylla[a], *P. thouarsii*[b]	11000000000000001000001201020100 01???0011201001031110 0
Pleiocarpa mutica	01000010000000001000011100300030013000002100200 1?1100
Plumeria rubra	01100001000000001001000200020020201000002000011010120 1
Pteralyxia kauaiensis[a,b], *P. macrocarpa*[b]	01100000000000001000001110100202 01???0111221001032200 0
Rauvolfia vomitoria	01200000000000002110101010000000112000102000320001110 0
Rhazya stricta	21100000000000002110001200010021212000002000021001120 0
Tabernaemontana divaricata[a], *T. pandacaqui*[b]	01000100001000001001001000300132114000002000 20?0211200
Thevetia peruviana	01100101100110120111001113200202001001020000 0?0101230
Vallesia antillana[a,b], *V. glabra*[b]	01100000000000001100001110300 02--03000003030000001120 0
Vinca major[b], *V. minor*[a,b]	21000000000000012110201200100120012000102402300100120 0
Gelsemium sempervirens	10010001020000 2-0---00120002002011200000020000000000250
Geniostoma rupestre	00010201020000000000000201300120 11???0002310101010110 0

Appendix 4.

Characters and character states for the morphological matrix used in the cladistic analyses. See Appendix 3.

The characters and character states used in this study are based on the exemplar method; only the characteristics of the species used in the analysis are considered in assigning codes. In cases of large genera with a range of states, this is indicated here.

1. Habit: 0 = trees or shrubs; 1 = lianas or vines; 2 = perennial herbs. The species used to represent *Alyxia* here, 4. *ruscifolia*, is a shrub; the great majority of *Alyxia* species, however, are lianas.

2. Non-articulated laticifers: 0 = absent; 1 = present. Non-articulated laticifers are one of the key characters that distinguish Apocynaceae s.l. from other Gentianales.

3. Phyllotaxis: 0 = leaves opposite; 1 = leaves alternate; 2 = leaves verticillate. Some taxa have leaves that are predominantly verticillate but may have some nodes with only two leaves. These taxa are coded as verticillate here.

4. Stipules: 0 = absent; 1 = present. Apocynaceae are here considered to be estipulate in the sense of Cronquist (1981) and Rosatti (1989). The colleters or interpetiolar ridges found in some taxa are not considered to be homologous with true stipules, nor are the adaxial outgrowths at the base of the petiole in *Alstonia scholaris* (Sidiyasa, 1998). Small bract- or scale-like organs that are found in some species of *Rauvolfia* have sometimes been called stipules. In a recent revision of the Neotropical species, however, Koch (2002) argued convincingly that these organs are better interpreted as cataphylls.

5. Supernumerary bracteoles: 0 = absent; 1 = present. Supernumerary bracteoles are clusters of bracteoles on the pedicel subtending the calyx. These bracteoles often resemble the sepals.

6. Calycine colleters: 0 = absent; 1 = several, across the inner face of the sepal (these sometimes coalesced at the base); 2 = few, mostly in the sepal sinuses. Calycine colleters are a common feature in Gentianales, and their lack or presence and arrangement is often used in Apocynaceae as an aid in distinguishing genera (e.g., Stapf, 1902; Woodson, 1933; Rosatti, 1989: 338–339; Ezcurra et al., 1992: 9–10; Omino, 1996: 87–88; Middleton, 1999, fig. 1). In *Alyxia ruscifolia*, although colleters are lacking at the base of the sepals themselves, they are well developed in the many supernumerary bracteoles clustered below the calyx. In Endress et al. (1996), *Plumeria* was coded as having a continuous row of calycine colleters. Detailed examination, however, has shown that they are not homologous to typical calycine colleters in that the entire inner surface of the upper part of the sepal is glandular. Because no other taxon shares this condition in this study, it is non-informative and thus not coded here.

7. Fusion of corolla tube: 0 = fused just above the level of stamen insertion; 1 = unfused (with gaps) just above the level of stamen insertion. In Apocynaceae, the lower corolla tube (composed of the united petal and stamen primordia) is congenitally fused; the upper part fuses postgenitally and basipetally, so that the last region to fuse is just above the insertion of the stamens. In some genera, flowers reach anthesis before fusion of the upper corolla is complete, resulting in gaps in the corolla tube (Boke, 1948; Nishino, 1982; Erbar, 1991). These gaps are especially long in *Aspidosperma*, *Geissospermum*, *Haplophyton*, and *Stephanostegia*, resulting in conspicuous slits that are easily visible with a dissecting microscope (Woodson, 1951; Fallen, 1986; Leeuwenberg, 1997). Shorter gaps of some microns in length (visible in microtome serial sections) are also found in *Alstonia*, *Craspidospermum*, and Hunterieae, whereas in other genera epidermal remnants are still visible, although there are no distinct gaps. In Endress et al. (1996), epidermal remnants and gaps were treated together as a single character state: corolla incompletely fused. Because, however, the tube may be fused yet still show epidermal remnants, here only the presence of distinct gaps, visible with dissecting microscope or in serial sections, is coded as unfused.

8. Corolla tube mouth: 0 = constricted; 1 = not constricted.

9. Infrastaminal appendages: 0 = absent; 1 = present. Infrastaminal appendages is a term used by Pichon (1948b) for outgrowths of the lower, congenitally fused part of the corolla tube in the staminal sectors (see Alvarado-Cárdenas & Ochoterena, 2007). They are found mainly in taxa previously included in Cerbereae (e.g., *Cerbera*, *Thevetia*, *Cerberiopsis*). These genera have a long, thin style and a disproportionately large, broad style head.

10. Corolla lobe aestivation: 0 = sinistrorsely contort; 1 = dextrorsely contort; 2 = imbricate. Corolla lobe aestivation is one of the most important morphological characters in Apocynaceae. With a few exceptions, the direction is constant within a genus. In Rauvolfioideae corolla lobes are almost always sinistrorsely contort, whereas in Apocynoideae, corolla lobes are normally dextrorsely contort or, rarely, valvate. The corolla lobes in *Kopsia*, *Ochrosia*, and *Neisosperma* (all Rauvolfioideae) are consistently dextrorsely contort (Hendrian, 2004; Middleton, 2004) and thus an exception to the rule. *Alstonia* is one of the few genera in the family in which both sinistrorsely as well as dextrorsely contort species occur, and this feature is constant only at the species level.

11. Petals in bud: 0 = not inflexed; 1 = inflexed. In most Apocynaceae, contorted petals in bud are spiraled upward into a tip. Petals that are inflexed in bud are, instead, folded downward and spiral into the mouth of the corolla tube and only unfold at anthesis. Inflexed petals is a relatively uncommon condition in Apocynaceae.

12. Corolline corona below petal sinus, behind and/or just above anther: 0 = absent; 1 = a compact protruding lobe. All outgrowths in the staminal sector and above the insertion of the anther are interpreted here as a corona. These include the vertical ridges in *Molongum* Pichon, as well as the protuberances termed suprastaminal appendages by Pichon (1948b) in *Thevetia*; the fimbriate lobes of *Allamanda* are also interpreted as a corona (see Endress et al., 1996).

13. Anthers: 0 = atop filaments that arise from the corolla tube; 1 = sessile upon enlarged staminal ridges.

14. Lignified guide rails: 0 = absent; 1 = present. Lignified guide rails are a specialization of the lateral parts of the anther and have a function in the complex pollination mechanism in Apocynaceae; they are absent in most Rauvolfioideae, but are characteristic for Apocynoideae. It is important to note that lignified guide rails are also characteristic for the majority of *Tabernaemontana* species (including all of the Neotropical taxa), although absent in the two representative species used in this study and in all of section *Ervatamia* to which they belong (see Leeuwenberg, 1994b: xv). Thus, for this character, most species of *Tabernaemontana* would show more affinity morphologically to *Molongum* than is apparent from the representative species used here (compare with Endress et al., 1996, in which a Neotropical species was used in the morphological analysis).

15. Anther dehiscence: 0 = introrse; 1 = latrorse; 2 = extrorse.

16. Anther/style head synorganization: 0 = anthers situated above or below, but not closely synorganized with, the style head; 1 = anthers at about the same level as, and connivent over and encircling the style head; 2 = anthers agglutinated to the style head via hair pads and adhesive. Synorganization of the anthers and the style head has always been a key character in Apocynaceae. It is the most important traditional character that separates Apocynoideae (in which the anthers are postgenitally united with the style head) from Rauvolfioideae (in which the anthers are free from the style head).

The lack of close synorganization of the anthers and style head in *Tabernaemontana divaricata* is not typical of the whole genus (as defined by Leeuwenberg, 1991). In all Neotropical species of *Tabernaemontana* and in some Paleotropical ones as well, the style head and anthers are more closely synorganized and would be coded as character state 1 in this study.

17. Style apex specialization: 0 = style apex without secretory epithelium; 1 = style apex transformed into an enlarged style head with epithelium of the body uniformly secretory and receptive; 2 = style apex transformed into an enlarged style head with epithelium of the body vertically differentiated with stigmatic region at base; 3 = style apex transformed into an enlarged style head, with epithelium of the body vertically differentiated, stigmatic zone at base, and radially mechanically interrupted by the adnate anthers. All Apocynaceae are characterized by having the carpel apices forming an enlarged style head with secretory epithelium. The degree and manner of histological differentiation of the style head and the epithelium is variable, with a specific type often characteristic of a particular tribe (Schick, 1980; Fallen, 1986). Although the gynoecium apex in *Geniostoma* J. R. Forst. & G. Forst. is post-genitally fused and enlarged, it is not covered with a secretory epithelium like that found in Apocynaceae. Instead, on male flowers, enlarged glue-filled irritable hairs with an abscissable tip are found scattered among the more numerous smaller, normal papillae (Endress et al., 1996). Specialized glue hairs like those found in *Geniostoma* are unknown in Apocynaceae.

18. Style head upper hair wreath: 0 = absent; 1 = present. Some style heads have a wreath of longer hairs just below the unfused carpel tips. The main function of the wreath is for pollen deposition and secondary presentation. The flowers are protandrous; shortly before anthesis, the anthers dehisce and shed their pollen toward the center of the flower. If the style head has an upper wreath, the pollen is shed onto this ring of hairs, which plays a role in the complex pollination mechanism of Apocynaceae (Church, 1908; Schick, 1980; Fallen, 1986).

19. Style head base: 0 = without collar or flange; 1 = with a distinct, thin collar; 2 = with a wreath of longer hairs; 3 = with thick flange. The base of the style head is often equipped with a means of scraping off donor pollen from the proboscis of an insect visitor. The presence (or absence) and type of scraper is often diagnostic of a particular tribe, and thus a useful character in the family. When a scraper is present at the base of the style head, the receptive zone is located beneath it (Schick, 1980; Fallen, 1986).

20. Style head unfused apices: 0 = small, inconspicuous, less than 1/3 the length of the total style head; 1 = enlarged, conical and tapering to blunt and clavate, at least 1/3 the length of the total style head.

21. Free disc nectary: 0 = absent; 1 = entire, annular; 2 = two separate lobes. In Apocynaceae, a free nectar disc is often present. Sometimes the nectar disc is adnate to the base of the ovary. Some taxa (especially in Rauvolfioideae) are nectarless and apparently use deceit pollination (Haber, 1984; Lin & Bernardello, 1999). In some cases it is difficult to distinguish whether or not the base of the ovary is nectariferous. For this reason, only the presence versus absence of a distinct nectary disc is coded here. In the large genus *Alstonia*, this character varies from species to species. The species included in this study, *A. scholaris*, has a shallow nectar disc. In some other species of *Alstonia*, a slight thickening can be discerned at the base of the ovary, and in yet others there is no indication of a nectary at all.

22. Ovary: 0 = 2-carpellate; 1 = 3–5-carpellate. Throughout Apocynaceae s.l., the gynoecium is composed of two carpels. The only exceptions are found in *Lepinia* and *Lepiniopsis* in the Alyxieae and in *Pleiocarpa* in Hunterieae (Endress et al., 1997).

23. Placentas: 0 = lignified or indurated in fruit; 1 = not lignified or indurated in fruit.

24. Mesocarp consistency: 0 = fleshy, without fibers; 1 = fleshy, with fibers; 2 = dry or woody.

25. Endocarp: 0 = not forming a stone around the seed; 1 = lignified or sclerified and forming a stone around the seed.

26. Seeds: 0 = sessile; 1= funiculate.

27. Seed shape: 0 = broad, compressed, not folded, mostly circular to ovoid; 1 = cylindrical, as if longitudinally rolled; 2 = narrowly fusiform, flattened, with a longitudinal fold; 3 = irregularly shaped, globular or angular, not flattened, or flattened on one side only, the other side convex.

28. Seed margin: 0 = with neither flattened edge nor wing; 1 = with a narrow flattened edge, this sometimes dissected; 2 = with a well-developed, usually membranous wing(s); 3 = fimbriate.

29. Seed coma: 0 = absent; 1 = present. A coma is a tuft of hairs all arising from a small restricted region at the end(s) of a seed. It is not considered to be homologous to the fimbria that are found around the margin of the seed in, for example, *Alstonia*.

30. Hilar depression: 0 = absent; 1 = an ovate depression, less than 50% the length of the seed; 2 = a deep, broad furrow, traversing the entire seed length; 3 = a deep, narrow fissure, traversing 75%–80% of the length of the seed.

31. Hilum shape: 0 = linear, traversing the length of the seed; 1 = linear, but shorter than the seed; 2 = small, circular (punctiform); 3 = ovate, covering a larger area.

32. Endosperm: 0 = not ruminate; 1 = with shallow, irregular tubercules or ruminations; 2 = with deep longitudinal ruminations. Ruminate endosperm, although relatively rare in Apocynaceae, is characteristic for *Tabernaemontana* and is also found in several genera of Alyxieae. *Chilocarpus* is unusual in this aspect in that the genus can be divided into two groups: those with smooth and those with ruminate endosperm (Pichon, 1949c; Markgraf, 1971). The representative species used in the analysis here belongs to the group with smooth endosperm; had a species from the other group been selected, *Chilocarpus* would fit better with other Alyxieae as to this character.

33. Endosperm: 0 = tough and corneous to subcartilaginous; 1 = firm, fleshy or starchy; 2 = delicate, soft or mealy. There is considerable variation in the thickness of the endosperm. For example, the endosperm of *Alyxia*, *Chilocarpus*, *Condylocarpon*, *Lepinia*, *Lepiniopsis*, and *Pteralyxia* is especially thick and tough (even difficult to cut with a razor blade). In *Allamanda*, *Picralima*, *Plectaneia*, and *Pleiocarpa*, endosperm is also tough but much thinner, but because no

clear demarcation between "thick" and "thin" could be found, only the consistency of the endosperm is coded.

31. Cotyledon base: 0 = auriculate; 1 = not auriculate. Although cotyledons are typically auriculate at the base in Tabernaemontaneae (sensu Leeuwenberg, 1991), this was not the case in the species included here.

35. Secoiridoids and complex indole alkaloids: 0 = absent; 1 = secoiridoids present, indole alkaloids absent; 2 = dominant indole alkaloids present, but only those with non-rearranged secologanin skeleton; 3 = dominant indole alkaloids present, including those with rearranged secologanin skeleton of the eburnan and/or plumeran type; 4 = dominant indole alkaloids present, including those with rearranged secologanin part of the ibogan type.

36. Cardenolides: 0 = absent; 1 = present.

37. Steroidal alkaloids: 0 = absent; 1 = present.

38. Pollen unit: 0 = monad; 1 = tetrad. Tetrads are rare in Rauvolfioideae, and of the taxa sampled here they occur only in *Condylocarpon* and *Craspidospermum*.

39. Pollen grain: 0 = small (≤ 51 µm); 1 = large (≥ 60 µm). Average largest pollen grain size (either the length of the polar axis, P, or the diameter of the equatorial plane, E) varies between 25 and 90 µm. It appears that a relatively large gap exists between 51 and 60 µm and that only *Anechites* (56 µm) falls between. Coding pollen size either as small (≤ 51 µm) or as large (≥ 60 µm), with one ambiguous case, gives two rather well-separated size classes.

40. Pollen grain shape: 0 = regular; 1 = irregular. Pollen grains with a regular shape have a zonoaperturate aperture system with equally spaced apertures on the equator. The polar axis and the equatorial plane can be easily indicated. In pollen grains with an irregular shape, the position/orientation of the polar axis and the equatorial plane cannot be indicated because there are only one or two porate apertures that are unequally spaced and sized and have an oblique orientation. Irregular pollen grains with three porate apertures have unequally spaced and sized apertures with oblique orientations.

41. Aperture number: 0 = zero; 1 = one or two; 2 = three or four; 3 = five or more. In Rauvolfioideae, only *Condylocarpon* has inaperturate pollen (aperture number = zero). Two-aperturate pollen (sometimes mixed with 1-aperturate) occurs in *Alyxia*, *Chilocarpus*, *Plectaneia*, and *Pteralyxia*, while 3- and/or 4-aperturate pollen (often mixed within a single sample) is found in most other genera. A few genera have five or more apertures (up to 10 apertures are found in *Craspidospermum*). In most samples studied, minor percentages of pollen grains with deviating aperture numbers are found, which is a common phenomenon in dicots. The coding given is for the dominant aperture numbers.

42. Ectoapertures: 0 = long colpi; 1 = short colpi; 2 = large pori; 3 = small pori; 4 = indistinct. Colpate ectoapertures are either longer than ca. 2/3 (long colpi) or shorter than ca. 1/3 (short colpi) the length of the polar axis (P). Large pori are at least 6 µm; if 6 µm (*Chilocarpus*, *Plectaneia*), then they are always accompanied by larger pori (up to 9 and 12 µm, respectively) in the same pollen grain. Small pores are 2–5 µm and do not vary much in size in a single grain. Due to its thin outer exine, *Vinca* pollen has indistinct ectoapertures.

43. Ectoaperture margin: 0 = not outwardly thickened; 1 = outwardly weakly thickened; 2 = outwardly distinctly thickened; 3 = with conspicuous arcus-like ridges. The ectoaperture margin is usually not thickened. In genera with large pores, but also in the brachycolpate *Molongum*, it is distinctly thickened into a well-delimited, protruding margin (aspidate pollen). In the genera with small pores, the ectoaperture margin is not or only weakly thickened. *Aspidosperma* and *Vallesia* have conspicuous ridges (massive and partly hollow, respectively) along the colpi joining toward the poles.

44. Endoapertures: 0 = distinct from and smaller than ectoapertures; 1 = not distinct from ectoapertures; 2 = distinct from and larger than ectoapertures. In *Alyxia*, *Lepinia*, *Lepiniopsis*, *Plectaneia*, and *Pteralyxia* (all with porate pollen), the endoapertures are not delimited from the ectoapertures (endo- and ectoapertures congruent). In other porate genera, the endopore is distinct by being situated in a differentiated inner exine layer, and also in all other taxa the endo- and ectoapertures are incongruent. In colporate pollen grains, the endoapertures are always smaller than the ectoapertures, except in *Vinca*, in which the endoapertures (delimited by costae) are larger than the ectoapertures.

45. Endoaperture margin: 0 = not inwardly thickened; 1 = with endoannulus; 2 = with polar costae; 3 = with lateral costae. This character can only be assessed by using LM and/or SEM images of the inner pollen-wall surface. An endoannulus is an inward thickening encircling the endoaperture. Polar costae are thickenings at the polar sides of usually lalongate endoapertures. Lateral costae are thickenings at the lateral sides of circular to lalongate endoapertures.

46. Supplementary endocolpi: 0 = absent (no endoplates recognizable); 1 = weak (zones of endocracks; endoplates indistinct); 2 = distinct (endoplates well recognizable). Supplementary endocolpi are narrow (e.g., *Cabucula*) to wide (e.g., *Rhazya*) zones along the colpi (one at each side) where the inner exine layer is more or less missing. They may join interaperturally toward the poles. In some genera (e.g., *Catharanthus*), they seem to have taken over at least some of the function of the ectocolpi, bordering on distinctly thicker intine parts (see El-Ghazaly, 1990, fig. 17). Usually, supplementary endocolpi have a granular inner surface and delimit smooth endoplates in the mesocolpium centers (mesocolpial plates) and under the colpi at the polar sides of the endoapertures (colpal plates). Supplementary endocolpi occur only in colporate genera (not all) and are absent in all porate genera.

47. Intine protrusions: 0 = absent; 1 = present. Coding of this character is largely based on data provided by Pichon (1947c, 1948a, b, c, 1950a, b). Protruding intine bulges at the endoapertures occur in both genera with porate pollen and genera with colporate pollen. In some cases, it could be observed (TEM) that the outer zone of a bulge has an intricately channeled structure. In *Alyxia*, there seems even to be a kind of relatively rigid operculum topping the protrusion (Huang, 1986).

48. Exine: 0 = not reduced; 1 = reduced (thin). *Condylocarpon* and *Vinca* have a thin exine (0.1–0.6 µm and ca. 0.1 µm, respectively), whereas in the other genera, exine thickness is at least 0.9 µm, but is usually much thicker.

49. Inner exine surface: 0 = psilate; 1 = scabrate; 2 = verrucate; 3 = granular. This character codes for the inner ornamentation of the exine (nexine surface) and was taken from the inside of the mesocolpia (mesoporia) centers. A scabrate surface has elements smaller than 1 µm. Verrucate and granular elements are larger than 1 µm, the former with a broad base, the latter with a constricted base.

50. Inner exine layer (nexine): 0 = ectexinous/endexinous (foot layer/endexine); 1 = ectexinous (endexine absent); 2 = endexinous (foot layer absent). This character codes for the composition of the inner exine layer (nexine).

Endexinous parts are indicated by 'white lines,' a lamellate structure and/or a ± spongy aspect. Ectexinous parts are ± homogeneous. Usually endexinous and ectexinous parts differ in contrast.

51. Infratectum: 0 = columellate; 1 = granular, reticulate or irregular; 2 = not recognizable (commissural line). A columellate condition is found only in *Gelsemium*. In most other genera, the infratectum is granular, reticulate or irregular, and varying in thickness. In *Alyxia*, *Lepinia*, *Lepiniopsis*, and *Pteralyxia*, an infratectum is indistinct (sparse gaps in inner exine layer), the contact between ectexine and endexine being largely a commissural line.

52. Tectum: 0 = thicker than infratectum + inner exine layer; 1 = equal to infratectum + inner exine layer; 2 = thinner than infratectum + inner exine layer. This character codes for the thickness of the tectum compared with the rest of the exine (infratectum + inner exine layer). It is also an approximate measure for the relative position of the infratectum. Usually the tectum is well delimited. When the boundary is irregular (e.g., in *Plectaneia*), the average tectum thickness was measured.

53. Outer exine surface: 0 = psilate (even to undulate); 1 = verrucate, with angular anastomosing verrucae; 2 = verrucate, with ± circular isolated verrucae; 3 = microreticulate; 4 = scabrate; 5 = striate-reticulate. This character codes for the outer ornamentation of the exine (tectum surface). Most genera have psilate pollen (no protuberances), with an even to undulate, often perforate surface. *Lepinia* and *Lepiniopsis* have verrucate pollen with anastomosing verrucae. The other states, except for scabrate exine, occur in single genera.

54. Mesocolpium/mesoporium centers: 0 = outer surface hardly or not deviating from surrounding exine; 1 = outer surface clearly deviating from surrounding exine. In about 1/4 of the sampled genera with colporate pollen, the outer surfaces of the mesocolpium centers have a different ornamentation compared with the surrounding areas. Usually the mesocolpium centers have a rugulate, microfossulate to verrucate, or a less distinctly perforate ornamentation.

PHYLOGENETIC RELATIONSHIPS BETWEEN *HOYA* AND THE MONOTYPIC GENERA *MADANGIA*, *ABSOLMSIA*, AND *MICHOLITZIA* (APOCYNACEAE, MARSDENIEAE): INSIGHTS FROM FLOWER MORPHOLOGY[1]

Livia Wanntorp[2,3] *and Paul I. Forster*[4]

Abstract

The monotypic genera *Absolmsia* Kuntze, *Madangia* P. I. Forst., Liddle & L. M. Liddle, and *Micholitzia* N. E. Br. (Apocynaceae, Marsdenieae) are, according to molecular phylogenetic results, nested within the genus *Hoya* R. Br. Together with *Dischidia* R. Br., these genera are considered to comprise a well-supported clade within the tribe Marsdenieae. Flower morphology of *Absolmsia*, *Madangia*, and *Micholitzia* is examined by SEM and light microscopy and compared to that of the related *H. caudata* Hook. f., *H. curtisii* King & Gamble, *H. hypolasia* Schltr., *H. lacunosa* Blume, *H. patella* Schltr., *H. retusa* Dalzell, and *H. venusta* Schltr. We discuss the morphological evidence that supports the reduction of these genera into *Hoya*. Among characters supporting such a position is the presence of a pellucid margin on the pollinia of *Absolmsia*, *Madangia*, *Micholitzia*, and *Hoya* that is absent on the pollinia of *Dischidia*. *Absolmsia* and *Madangia* have a staminal corona with outer and inner lobes, similar to that of *Hoya*. While the outer corona processes of *Absolmsia* and *Micholitzia* are free and revolute, as in most species of *Hoya*, those of *Madangia* are laterally fused forming a continuous skirt resembling the corona of certain species of *Hoya*. The flowers of *Micholitzia* are superficially similar to those of *Dischidia* in its urceolate corolla, but the staminal corona with revolute outer processes decidedly links *Micholitzia* to *Hoya*. The new combination *H. inflata* (P. I. Forst., Liddle & L. M. Liddle) L. Wanntorp & P. I. Forst. is proposed. For *Micholitzia obcordata* N. E. Br., the name *H. yuennanensis* Hand.-Mazz. has nomenclatural priority and should therefore be used when including *M. obcordata* in *Hoya*.

Key words: *Absolmsia*, Apocynaceae, corolla, corona, *Dischidia*, *Hoya*, *Madangia*, Marsdenieae, *Micholitzia*, morphology, pellucid margin, phylogeny, pollinaria, pollinia.

The tribe Marsdenieae Bentham (1868) (Apocynaceae, Asclepiadoideae) is thought to include 29 genera and over 570 species distributed in the New and the Old Worlds (Omlor, 1998). The taxonomic positions of the genera *Cionura* Griseb., *Dregea* E. Mey., *Dischidia* R. Br., *Gymnema* R. Br., *Hoya* R. Br., *Marsdenia* R. Br., *Micholitzia* N. E. Br., *Rhyssolobium* E. Mey., *Stephanotis* Thou., and *Telosma* Coville have been examined in previous morphological and molecular phylogenetic studies (Liede & Albers, 1994; Forster, 1995; Endress & Bruyns, 2000; Potgieter & Albert, 2001; Sennblad & Bremer, 2002; Rapini et al., 2003; Livshultz, 2003; Meve & Liede, 2004; Wanntorp et al., 2006a). However, only a few species of each of these genera were included in these studies, leaving several relationships within the Marsdenieae unresolved.

One of the genera in the Marsdenieae whose taxonomic delimitations remain unclear is *Hoya*, the wax plants of ornamental horticulture, a large tropical genus of over 300 herbaceous to succulent species of which about 40 are commonly found in cultivation (Forster et al., 1998, 1999, 2002), although many more are cultivated in specialist collections. Species of *Hoya* are distributed from mainland Asia (China, India, Vietnam, Burma, Laos, Cambodia) through Malaysia, Indonesia, and New Guinea to Australia and the western Pacific islands (Forster & Liddle, 1996). Most species of *Hoya* are laticiferous wiry climbers, often epiphytic, with fleshy to succulent leaves of

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various size, color, and venation. A minority of species are non-climbing and with foliage that is either erect or pendent. The sympetalous flowers are clustered in umbelliform extra-axillary inflorescences mostly with perennial peduncles (Forster et al., 1998). The complex, fleshy flowers of *Hoya* comprise a pentamerous corolla that is highly variable in shape, color, and dimensions; a staminal corona with inner processes adnate to the anther connective and outer processes with revolute lobes; a gynostegium, standing on a short or long column above the corolla and including five fused anthers, the ovaries, and the style head (Forster et al., 1998); and two pollinia from adjacent anthers, united by a translator apparatus secreted from the style head to a pollinarium (Omlor, 1998; Kleijn & van Donkelaar, 2001).

A morphological revision of the Marsdenieae placed *Hoya*, *Dischidia*, and the monotypic genera *Absolmsia* Kuntze, *Madangia* P. I. Forst., Liddle & I. M. Liddle, and *Micholitzia* together in a *Hoya*-group, sharing an epiphytic habit, fleshy to succulent leaves (in *Absolmsia* only present at an early age stage), generally persistent extra-axillary inflorescences, and comose and fusiform seeds without prominent wings but with keel-shaped thickenings on the seed coats (Omlor, 1998; Livshultz, 2003). In addition to Omlor's work, a possible close relationship between *Micholitzia*, *Madangia*, *Absolmsia*, and *Hoya* was discussed in morphological papers specific to each of these genera (Goyder & Kent, 1994; Forster et al., 1997; Kloppenburg, 2001). While the first two of these studies discussed the similarity of *Madangia inflata* P. I. Forst., Liddle & I. M. Liddle and *Micholitzia obcordata* N. E. Br. to *Hoya* without taking position regarding the relationship of these species to *Hoya*, Kloppenburg (2001) concluded that *A. spartioides* Kuntze should be included in *Hoya* based on its morphological similarities in the corolla and corona. Kloppenburg therefore proposed a change of name for the single species of *Absolmsia* to *H. spartioides* (Benth.) Kloppenb. None of these studies, however, examined relationships using a phylogenetic approach. Another species has been referred to the genus *Absolmsia*, namely *A. oligophylla*. This taxon was, however, recently shown to belong to the genus *Tylophora* R. Br. (Gilbert et al., 1995).

Recently, two molecular phylogenetic studies based on chloroplast gene regions of the *trn*L intron, *trn*L-*trn*F spacer, and *atp*B-*rbc*L spacer, as well as on nuclear sequences of the ribosomal ITS region, were conducted to examine the phylogeny of *Hoya* and its taxonomic delimitation (Wanntorp et al., 2006a, b). Thirty-five species of *Hoya*, three species of *Dischidia*, *Absolmsia spartioides*, *Madangia inflata*, and *Micholitzia obcordata* were analyzed, together with species representing subfamilies Periplocoideae and Secamonoideae, as well as tribes Fockeae, Marsdenieae, Ceropegieae, and Asclepiadeae (sensu Endress & Stevens, 2001; Verhoeven et al., 2003). A major result of these studies was that *Hoya* is paraphyletic without the inclusion of the monotypic genera *Absolmsia*, *Madangia*, and *Micholitzia*, which are all found clearly nested among the 35 species of *Hoya* examined (Wanntorp et al., 2006a, b).

The aim of the present study is to examine the flower morphology of *Absolmsia spartioides*, *Madangia inflata*, *Micholitzia obcordata*, and a selection of closely related species of *Hoya* by using light and SEM microscopy. We discuss which morphological characters bear on the phylogenetic and taxonomic positions of these genera within *Hoya*.

Material and Methods

We examined the floral morphology of the species *Absolmsia spartioides*, *Hoya caudata* Hook. f., *H. curtisii* King & Gamble, *H. hypolasia* Schltr., *H. lacunosa* Blume, *H. patella* Schltr., *H. retusa* Dalzell, *H. venusta* Schltr., *Madangia inflata*, and *Micholitzia obcordata*. Flower material at anthesis was collected and stored in 70% ethanol at the Botanical Department of Stockholm University, except for the material of *Madangia inflata* provided by D. J. Liddle (Queensland, Australia), of *Absolmsia spartioides* by T. Nyhuus (Järfälla, Sweden), of *H. curtisii* by A. Boström (Stockholm, Sweden), and of *H. hypolasia* and *H. venusta* by C. Forsberg-Heikkilä (Porvoo, Finland). The identity of all species examined was confirmed with the literature or by comparison with original type material. Table 1 reports voucher information for the specimens examined.

SEM and light microscopic studies were performed on all species mentioned above with the exception of *Hoya hypolasia* and *H. venusta*, which were only examined by SEM microscopy, due to scarce material. For SEM microscopy, all flowers were dehydrated in an alcohol-acetone series, critical-point dried, and sputter-coated with gold. SEM microscopy was done with a LEO 438 VP scanning electron microscope (Zeiss, Jena, Germany) at the Department of Systematic Botany, Ludwig Maximilians University, Munich. For sectioning, microtome sections were made using 2-hydroxyethyl methacrylate embedding (Igersheim & Cichocki, 1996), cut at 10 µm, and stained with toluidine blue and ruthenium red. When discussing the morphology of a single corona lobe, we use the term corona lobe, while we define the inner and outer parts of each lobe by using the terms inner (ip) and outer (op) processes, respectively.

Table 1. Voucher information on the specimens used in the present study. Herbarium abbreviations follow Index Herbariorum (Holmgren et al., 1990).

Species	Voucher information
Absolmsia spartioides Kuntze	*Wanntorp L. 592* (S), Sipitang, Borneo
Hoya caudata Hook. f.	*Wanntorp L. 593* (S), ex hort. Department of Botany, Stockholm University
Hoya curtisii King & Gamble	*Wanntorp L. 597* (S), ex hort.
Hoya hypolasia Schltr.	*Wanntorp L. 594* (S), ex hort.
Hoya lacunosa Blume	*Wanntorp L. 571* (S), ex hort. Department of Botany, Stockholm University
Hoya patella Schltr.	*Wanntorp L. 575* (S), ex hort. Department of Botany, Stockholm University
Hoya retusa Dalzell	*Wanntorp L. 580* (S), 1998–3127, Rosendal Uppsala, Uppsala Botanical Garden
Hoya venusta Schltr.	*Wanntorp L. 595* (S), ex hort.
Madangia inflata P. I. Forst., Liddle & I. M. Liddle	*D. J. Liddle IML 1076* (BRI), Madang Province, New Guinea
Micholitzia obcordata N. E. Br.	*Wanntorp L. 596* (S), ex hort.

Results

1. Hoya spartioides (Benth.) Kloppenb., Fraterna 14: 8. 2001. Basionym: *Astrostemma spartioides* Benth. in Hook., Ic. Pl. 14: 7. 1880. *Absolmsia spartioides* (Benth.) Kuntze, as "*spartoides*," Revis. Gen. Pl. 2: 418. 1891. TYPE: [Malaysia]. North Borneo, s.d., *Burbidge s.n.* (lectotype, designated here, K [1 of 2 (fl), 2 of 2 (st)] not seen).

Hoya spartioides is an epiphytic, sparsely branched, non-twining subshrub with fleshy leaves from Borneo. Inflorescences pedunculate, comprising concave umbels with 4 to 6 flowers; pedicels ca. 1 mm long, glabrous, rough. Calyx lobes obtuse-triangular, ca. 0.02 × 0.02 mm. Corolla shortly campanulate to salver-shaped, orange-yellow, glabrous, ca. 10 mm diam., with 5 triangular oval lobes, ca. 3 × 3 mm (Fig. 1A). Gynostegium on a short hairy column (Fig. 1C). Corona flat, star-shaped, white, smooth, covered by thick hairs on the upright part (Fig. 1A–C, E, G); inner processes of lobes narrow, spathulate, appressed to the anther appendages (Fig. 1D); outer lobes with deeply revolute processes (Fig. 1D, E); anther appendages lanceolate-acute, approximately as long as the corona lobes (Fig. 1F, G); style head flat (Fig. 1H). Pollinarium consisting of 2 pollinia connected by thickened winged caudicles to a corpusculum; pollinia square in outline, almost as long as wide, ca. 0.3 × 0.2 mm (Fig. 2E, G, H), the pellucid margin very wide but covering only ca. 1/3 of the dorsal margin of the pollinia (Fig. 2H); corpusculum thick, almost round in shape, ca. 0.2 × 0.2 mm, ending with 2 long, spreading appendages, upper part enclosing the caudicles, ca. 0.2 × 0.1 mm (Fig. 2F).

The herbarium specimen chosen as lectotype for *Hoya spartioides* was selected among the collections available for this plant at the Royal Botanic Gardens, Kew (D. Goyder, pers. comm., 2007).

2. Hoya caudata Hook. f., Fl. Brit. Ind. 4(10): 60. 1883. TYPE: [Malaysia]. "Malacca," *Maingay 1128* (holotype, K not seen).

Hoya caudata is an epiphytic twiner with leathery-fleshy leaves from the Malay Peninsula and Thailand. Inflorescences pedunculate, comprising geotropic, concave umbels, with up to 12 pendent flowers; pedicels longer in the periphery of the inflorescence and shorter in its middle, 2–3 cm long, hirsute. Calyx lobes linear-lanceolate, ca. 2 × ca. 1 mm, glabrous. Corolla flattened-campanulate, pink-yellow, inside pubescent in the middle, with long caudate hairs at the margins (Fig. 3A, B), ca. 2 cm diam.; lobes ovate-triangular, acute-cuspidate, 4–7 × 4–5 mm (Fig. 3A). Gynostegium stalked on a short column (Fig. 3A). Corona with a dark red ring in the middle but lighter at the margins, smooth; inner processes slightly pointing up (Fig. 3C); outer processes very wide, bending upward, not deeply revolute (Fig. 3C); anther appendages slender and whip-like, very much exceeding the corona lobes (Fig. 3B–D); style head long-apiculate (Fig. 3D). Pollinarium comprising 2 pollinia connected by broadly winged and flattened caudicles to a corpusculum (Fig. 3E); pollinia longer than wide, cylindrical-elongate, ca. 0.8 × 0.2 mm; pellucid margin covering the dorsal margin of the pollinia; corpusculum narrow-rhomboid, ca. 0.2 × 0.1 mm, ending with 2 long, spreading appendages (Fig. 3F); caudicles ca. 0.1 × 0.1 mm (Fig. 3E).

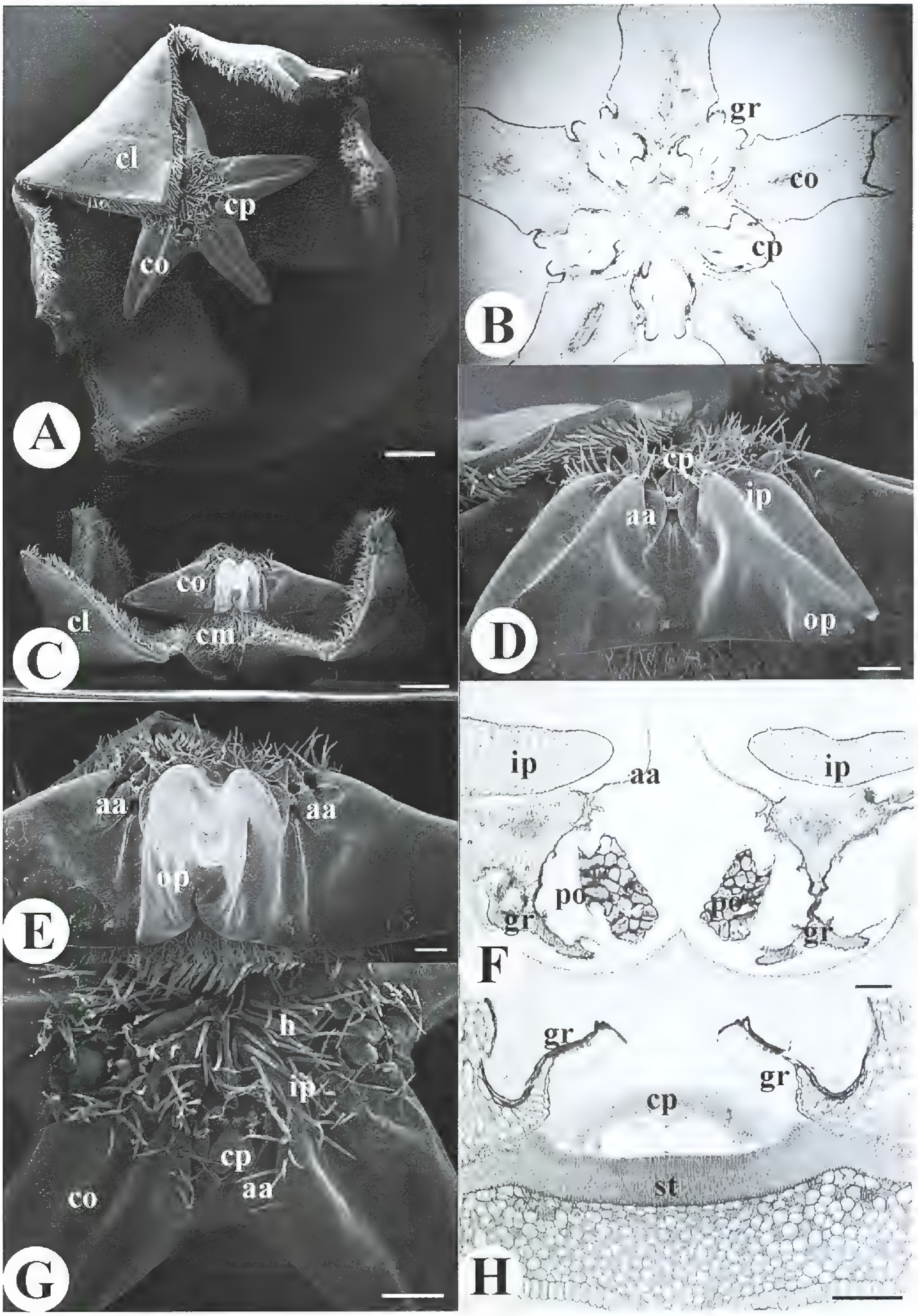

Figure 1. *Hoya spartioides*. —A, B. Top views of flowers showing remains of the corolla (cl), corona (co), guide rail (gr), and corpusculum (cp). —C. Side view of flower showing column (cm). —D, E. Details of coronas showing inner processes (ip), outer processes (op), anther appendages (aa), and corpusculum (cp). —F. Longitudinal section of flower showing the position of inner corona processes (ip), guide rail (gr), pollinia (po), and anther appendages (aa). —G. Detail of flower (top view) with corona (co) with hairs (h), anther appendages (aa), and corpusculum (cp). —H. Light micrographic section (longitudinal view) of flower showing the style head (st) and part of the corpusculum (cp). Scale bars = 1 mm (A, C), 4 mm (B), 100 µm (F, H), 200 µm (E), 300 µm (D, G).

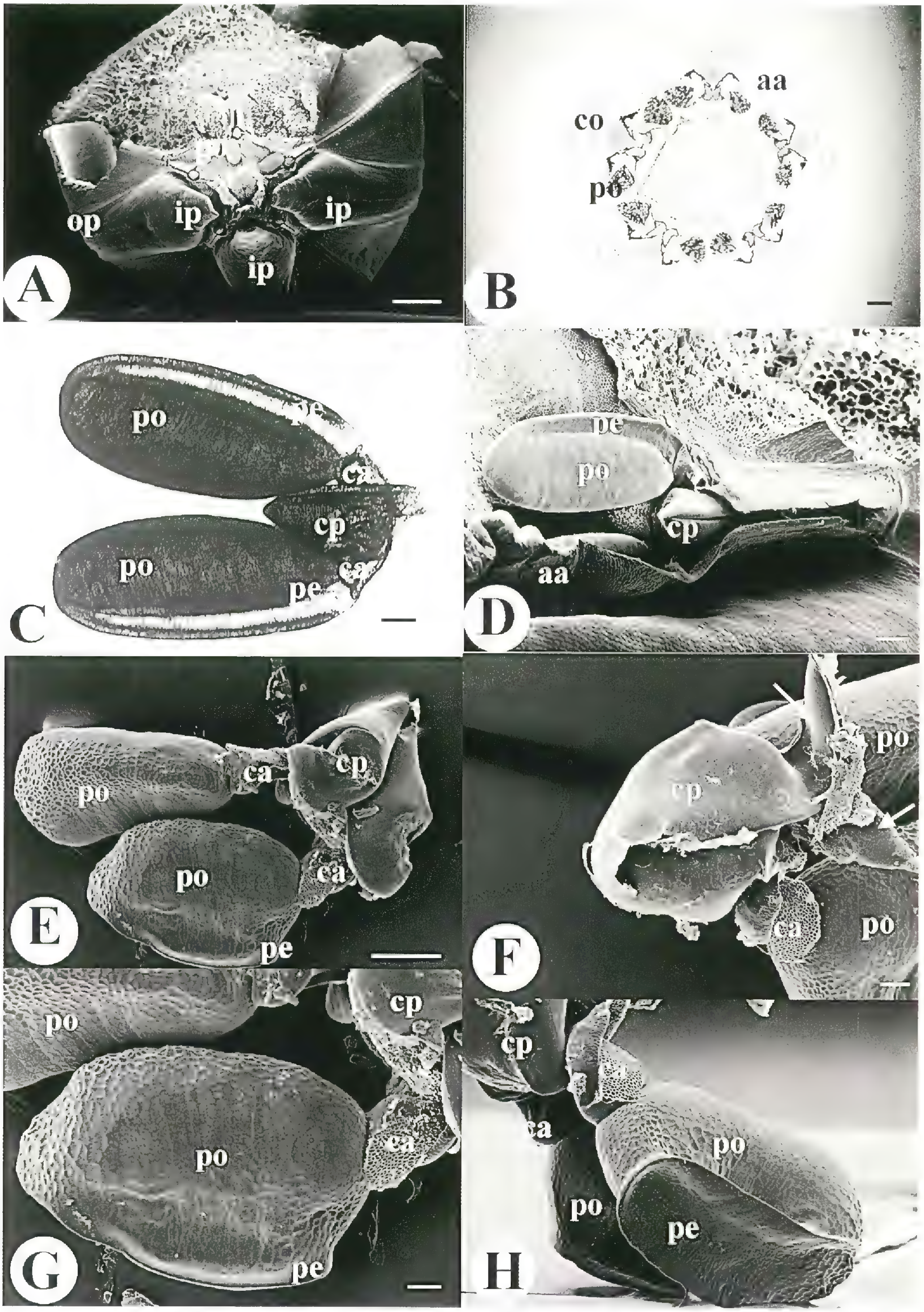

Figure 2. *Hoya inflata*. —A. Upper part of flower seen from above with inner (ip) and outer (op) corona processes, pollinia (po), and style head (st). —B. Cross section of flower showing the positions of coronas (co), anther appendages (aa), and pollinia (po). —C, D. Pollinaria with corpusculum (cp), caudicles (ca), and pollinia (po) with pellucid margins (pe) and one anther appendage (aa). Scale bars = 1 mm (A), 4 mm (B), 100 µm (C, D). *Hoya spartioides*. —E, G. Side view of pollinaria formed by the corpuscula (cp), the caudicles (ca), and the pollinia (po) with pellucid margin (pe). —F. Pollinarium seen from below. Arrows denote long appendages from the corpusculum partly covering the winged caudicles. —H. Pollinia (po) with broad pellucid margin (pe) and caudicles (ca). Scale bars = 30 µm (F–H), 100 µm (E).

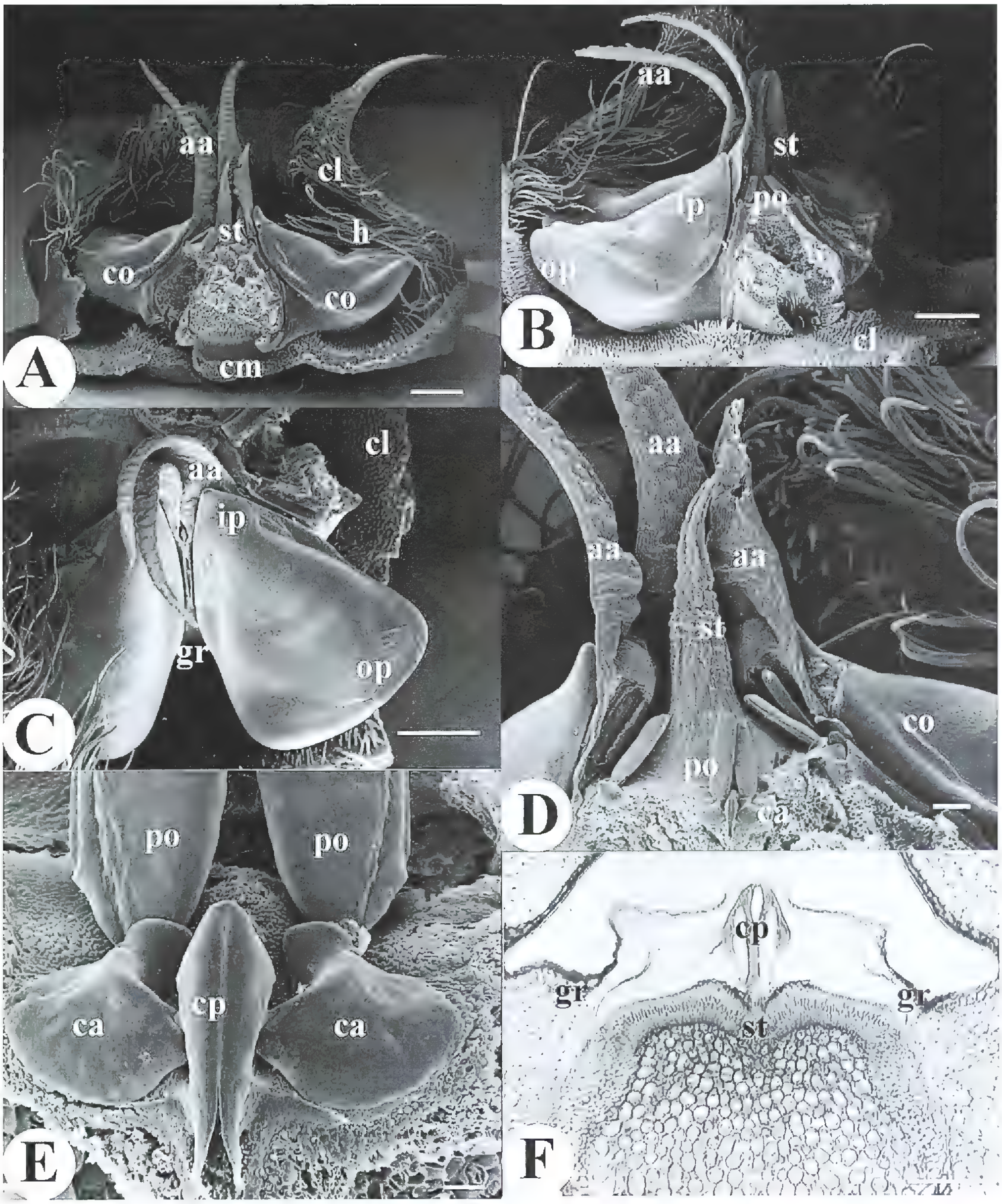

Figure 3. *Hoya caudata*. —A, B. Flowers showing remains of corolla (cl) with hairs (h), corona (co) with inner (ip) and outer (op) processes on a column (cm), anther appendages (aa), and style head (st). —C. Detail of flower showing guide rails (gr). —D. Upper part of flower with anther appendages (aa), style head (st), and pollinia (po) with falcate caudicles (ca). —E. Details of a pollinarium: pollinia (po), caudicles (ca), and corpusculum (cp). —F. Detail of flower showing the positions of guide rails (gr), corpusculum (cp), and style head (st). Scale bars = 1 mm (A–C), 200 µm (D), 30 µm (E), 100 µm (F).

3. Hoya curtisii King & Gamble, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 74(2): 563. 1907. TYPE: Malay Peninsula, *Curtis 2894* (holotype, SING!).

Hoya curtisii is an epiphyte with non-twining pendent stems and succulent leaves from the Malay Peninsula. Inflorescences pedunculate, comprising convex, globose umbels with up to 30 flowers; pedicels ca. 22 mm long, densely hairy. Calyx lobes narrowly triangular, ca. 2 × 1 mm. Corolla rotate, creamy white, inside completely pubescent, except on the margins, ca. 0.7 mm diam. (Fig. 4A); lobes oblong-oblanceolate, acute at the tips, reflexed, ca. 7 × 4 mm. Gynostegium borne on an annular structure (annulus) on a high column (Fig. 4A, B, D). Corona red in the middle and white at the margins; lobes sulcate, with a central depression or an umbo (Fig. 4A); inner processes blunt (Fig. 4E); outer

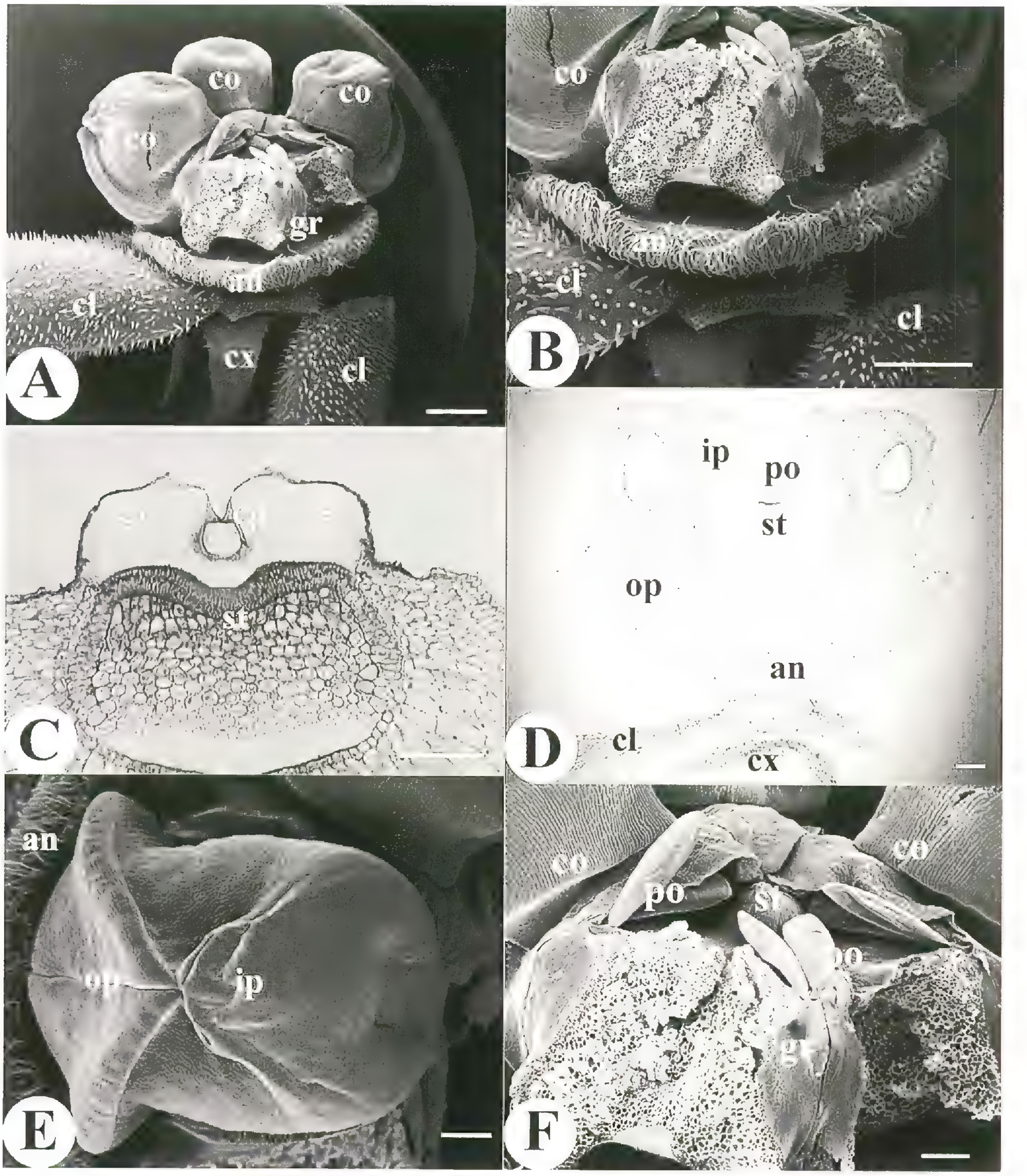

Figure 1. *Hoya curtisii*. —A, D. Flowers with calyx (cx), corolla (cl), corona (co) with inner (ip) and outer (op) processes, annulus (an), guide rails (gr), style head (st), and pollinia (po). —B. Enlargement of A showing the position of the annulus (an). —C. Part of flower showing guide rails (gr), corpusculum (cp), and style head (st). —E. Single corona lobe lying on annulus (an) with inner (ip) and outer (op) processes. —F. Part of flower showing coronas (co), guide rails (gr), pollinia (po), and style head (st). Scale bars = 1 mm (A, B), 2.8 mm (D), 100 µm (C), 300 µm (E, F).

processes made of 3 parts, of which 2 wide and bent against each other, and the third part bending upon these 2 and touching them (Fig. 4E); anther appendages lanceolate, blunt, exceeding the corona lobes (Fig. 5C); style head flat (Figs. 4C, F, 5C). Pollinarium comprising 2 pollinia attached by flattened and winged caudicles to a corpusculum; pollinia spindle-shaped, much longer than wide, ca. 10 × 0.3 mm (Figs. 4F, 5A, B, D); pellucid margin covering 1/2 of the dorsal margin of the pollinia (Fig. 5D); corpusculum ca. 1 × 0.3 mm, ending with 2 long, spreading appendages; caudicles ca. 0.1 × 0.04 mm (Fig. 5A, D).

4. Hoya hypolasia Schltr., Bot. Jahrb. Syst. 50: 123. 1913. TYPE: New Guinea. *R. Schlechter 18075* (holotype, B not seen).

Hoya hypolasia is an epiphytic twiner with fleshy leaves from New Guinea. Inflorescences pedunculate,

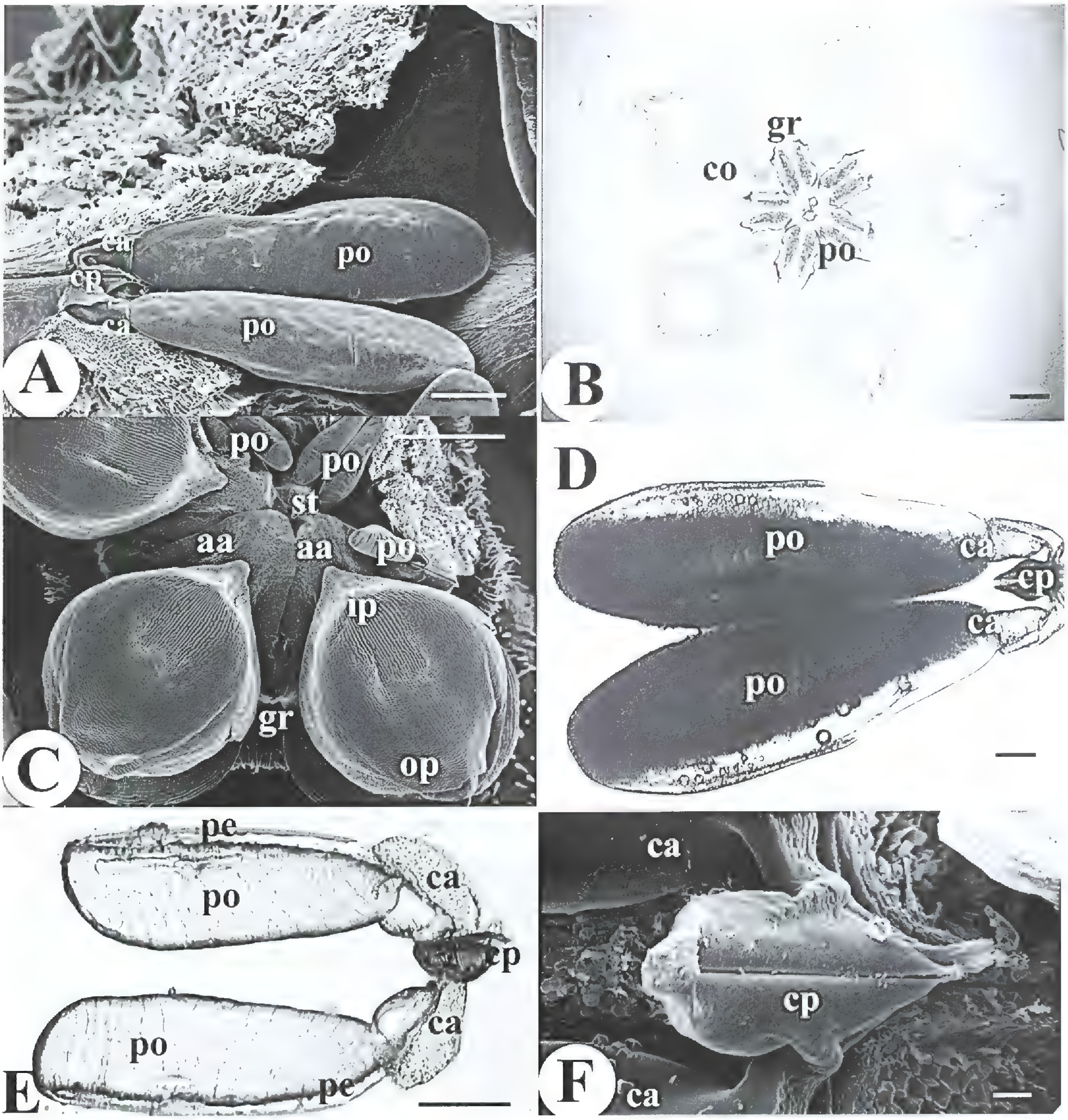

Figure 5. *Hoya curtisii*. —A, D. Pollinaria with pollinia (po), corpuscula (cp), and caudicles (ca). —B, C. Flowers (B, cross section), showing inner (ip) and outer (op) corona (co) processes, anther appendages (aa), guide rails (gr), pollinia (po), and style head (st). Scale bars = 1 mm (C), 2 mm (B), 100 μm (D), 200 μm (A). *Hoya lacunosa* —E. Pollinarium with corpusculum (cp), winged caudicles (ca), and pollinia (po) with pellucid margins (pe). —F. Detail of a pollinarium with corpusculum (cp) and part of caudicles (ca). Scale bars = 10 μm (F), 100 μm (E).

comprising concave umbels of ca. 10 flowers, ca. 2 cm diam.; pedicels ca. 40 mm long, glabrous. Calyx lobes obtuse, ca. 0.1 × 0.1 mm. Corolla flattened-campanulate, creamy white, outside glabrous, inside hairy, ca. 1.5 cm diam. (Fig. 6A, B, D); lobes ovate-triangular, ca. 0.8 × 0.6 mm. Inner processes of corona lobe very short and obtuse (Fig. 6F, H), not covering the anther appendages and the style head (Fig. 6C); outer processes revolute (Fig. 6F, H); anther appendages lanceolate, acute, exceeding the corona lobes (Fig. 6F); style head flat (Fig. 6C, D). Pollinarium comprising 2 pollinia attached by broadly winged and flattened caudicles to a corpusculum; pollinia oblong-elongate, basal part protruding outward, ca. 0.9 × 0.3 mm (Fig. 6E, G); pellucid margin covering the entire dorsal margin (Fig. 6G); corpusculum ca. 0.2 × 1 mm, ending with 2 short appendages (Fig. 6G); caudicles very short, ca. 0.1 × 0.03 mm (Fig. 6E, G).

5. Hoya lacunosa Blume, Bijdr. Fl. Ned. Ind. 16: 1063. 1826. *Otostemma lacunosum* (Blume)

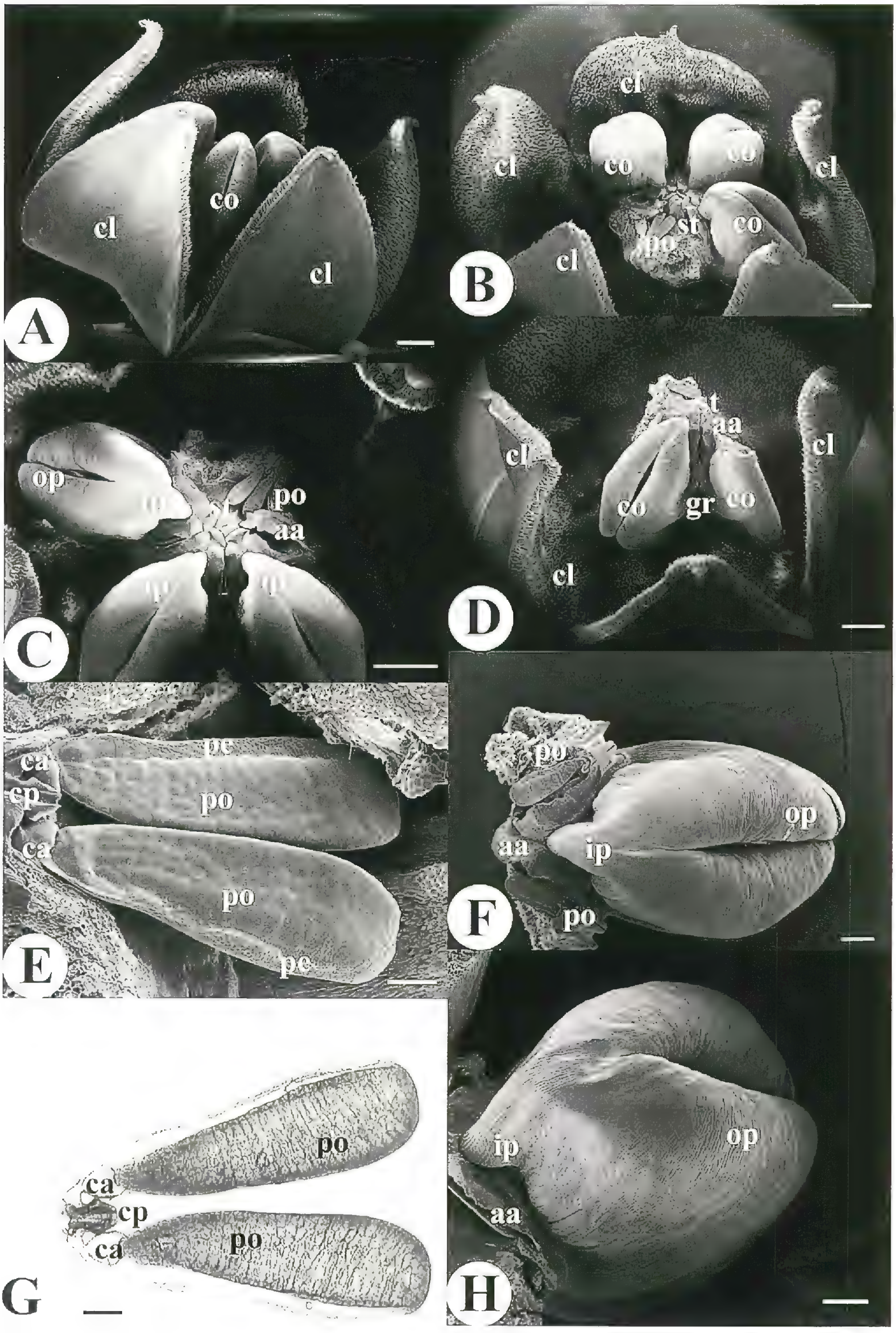

Figure 6. *Hoya hypolasia*. —A. Flower from side showing corollas (cl) and corona (co). —B–D. Parts of flowers seen from above with corolla (cl), inner (ip) and outer (op) corona (co) processes, guide rails (gr), anther appendages (aa), pollinia (po), and style head (st). —E, G. Pollinaria with pollinia (po), corpuscula (cp), caudicles (ca), and pollinia (po) with pellucid margins (pe). —F, H. Different views of single corona lobe with inner (ip) and outer (op) processes, anther appendages (aa), and pollinia (po). Scale bars = 1 mm (A–D), 100 µm (E, G), 300 µm (F, H).

Blume in Rumphia 4: 30. 1849. TYPE: Indonesia. *Horsfield s.n.* (holotype, K not seen).

Hoya lacunosa is a twiner from Thailand and the Malay Peninsula. Inflorescences pedunculate, comprising pendent, convex umbels, with up to 30 flowers, ca. 5 mm diam.; pedicels of outer flowers longer than central ones, 10–20 mm long, glabrous. Calyx lobes triangular, ca. 1 × 0.5 mm. Corolla rotate, white, ca. 5 mm diam.; lobes lanceolate, acute-cuspidate, totally reflexed giving the flower a globose shape, very hairy, ca. 4 × 2 mm (Fig. 7A). Gynostegium on a conspicuous column (Fig. 7G). Corona lobes elliptical, basal part forming a continuous structure looking like a skirt (Fig. 7B, D, F, G); inner processes acutely ascending, outer processes obtuse, slightly ascending (Fig. 7A–F); anther appendages lanceolate, acute, exceeding the corona lobes (Fig. 7F); style head shortly apiculate (Fig. 7E, H). Pollinarium comprising 2 pollinia attached by broadly winged and flattened caudicles to a corpusculum; pollinia elongate-falcate, ca. 0.4 × 0.1 mm (Fig. 5E); corpusculum ca. 0.1 × 0.1 mm, ending with 2 short appendages (Fig. 5E, F); caudicles thick, broadly winged, ca. 0.1 × 0.1 mm (Fig. 5E).

6. **Hoya inflata** (P. I. Forst., Liddle & I. M. Liddle) L. Wanntorp & P. I. Forst., comb. nov. Basionym: *Madangia inflata* P. I. Forst., Liddle & I. M. Liddle, Austrobaileya 5: 54. 1997. TYPE: Papua New Guinea. Madang Province: Headwaters Dom River, 26 Nov. 1995, *I. M. Liddle IML1076* (holotype, BRI!).

Hoya inflata is an epiphytic twiner with fleshy leaves from New Guinea. Inflorescences pedunculate, comprising pendent, convex umbel, with up to 9 flowers, ca. 1 × ca. 2 cm; pedicels sparsely hirsute, 30–45 mm long. Calyx lobes triangular, ca. 1 × 2 mm. Corolla urceolate-globose, fleshy, creamy white, basally hiding the calyx (Fig. 8D), glabrous on the outside, basal inside part covered by long, slender, woolly hairs, ca. 1 × 2 cm (Fig. 8A); lobes triangular, somewhat reflexed, ca. 5 × 6 mm. Gynostegium on a conspicuous hairy columnar structure (Fig. 8A). Corona made of 5 continuous lobes forming a membranous smooth skirt, whose lower part builds up a continuous rim that is bent inward (Fig. 8A, C–E), white; lobes free at the style head (Figs. 8E, F, 2A); anther appendages lanceolate, acute, protruding above the corona lobes (Fig. 8B, E); style head flat (Fig. 8B, F, G). Pollinarium comprising 2 pollinia connected by unwinged and flattened caudicles to a corpusculum; pollinia with oblong-round apical part, ca. 0.7 × 0.3 mm (Figs. 8G, 2B–D); pellucid margin covering the entire dorsal margin of the pollinia and slightly protruding outward at the basal part; corpusculum oblong-ovate, ca. 0.4 × 0.2 mm (Fig. 2C, D); caudicles ca. 0.1 × 0.7 mm (Fig. 2C).

7. **Hoya yuennanensis** Hand.-Mazz., Symb. Sin. 7(4): 1001. 1936. TYPE: China. Yunnan: betw. Ota-Tanschan & Tsedjrong, 4 Oct. 1915, *Handel-Mazzetti 7971* (holotype, W not seen).

Micholitzia obcordata N. E. Br., Bull. Misc. Inform. Kew 358. 1909. Syn. nov., non *Hoya obcordata* Hook. f., Fl. Brit. India 4: 56. 1885. *Dischidia obcordata* (N. E. Br.) J. F. Maxwell & R. van Donkelaar, Nat. Hist. Bull. Siam. Soc. 39: 78. 1991. TYPE: "India & Burma," cult. Royal Bot. Gard. Glasnevin, Dublin, 1909, *Micholitz s.n.* (holotype, K not seen).

Hoya lantsangensis Tsiang & P. T. Li, Acta Phytotax. Sin. 12: 126. 1974. Syn. nov. *Antiostelma lantsangense* (Tsiang & P. T. Li) P. T. Li, Novon 2: 218. 1992. TYPE: China. Yunnan: Mo-Jiang, Talang, *A. Henry 13589* [cited by Tsiang & P. T. Li as *A. Henry 13689*] (holotype, NY not seen; isotypes, IBSC not seen, K not seen).

Hoya manipurensis Deb, J. Indian Bot. Soc. 34: 50. 1955. *Antiostelma manipurense* (Deb) P. T. Li, Novon 2: 219. 1992. TYPE: India. Manipur: Litan, 1 Sep. 1953, *D. B. Deb 1081* (holotype, CAL not seen).

Hoya yuennanensis is an epiphytic vine with fleshy leaves from China (Yunnan), India, Burma, and Thailand. Inflorescences pedunculate, comprising racemes of up to 7 flowers, ca. 1.4 × ca. 0.8 mm; pedicels ca. 2 mm long, hirsute. Calyx lobes obtuse-triangular, ca. 0.2 × 0.3 mm, slightly pubescent. Corolla narrowly urceolate, greenish with reddish lobular margins uniformly densely pilose on the outside; tube wider at the base and gradually narrowing up to the rim, inside covered by uniformly distributed long hairs, ca. 1.2 × 0.6 mm (Fig. 9A); lobes triangular, slightly reflexed, inside densely pilose, ca. 0.2 × 0.1 mm (Fig. 9A). Corona with 5 fleshy sulcate lobes, white; inner processes short and slightly bent upward (Fig. 9C); outer processes with a deep groove (Fig. 9C, D); anther appendages much exceeding the corona lobes and ending with a triangular structure that touches the style head (Fig. 9A–C, E); style head short, conical. Pollinarium comprising 2 pollinia connected by flattened, unwinged caudicles to a corpusculum; pollinia lying in an almost horizontal position in regard to the corona, almost squared, ca. 0.2 × 0.2 mm (Fig. 9F, G); pellucid margin covering the entire dorsal margin of the pollinia (Fig. 9F, G); corpusculum rhomboid, ca.

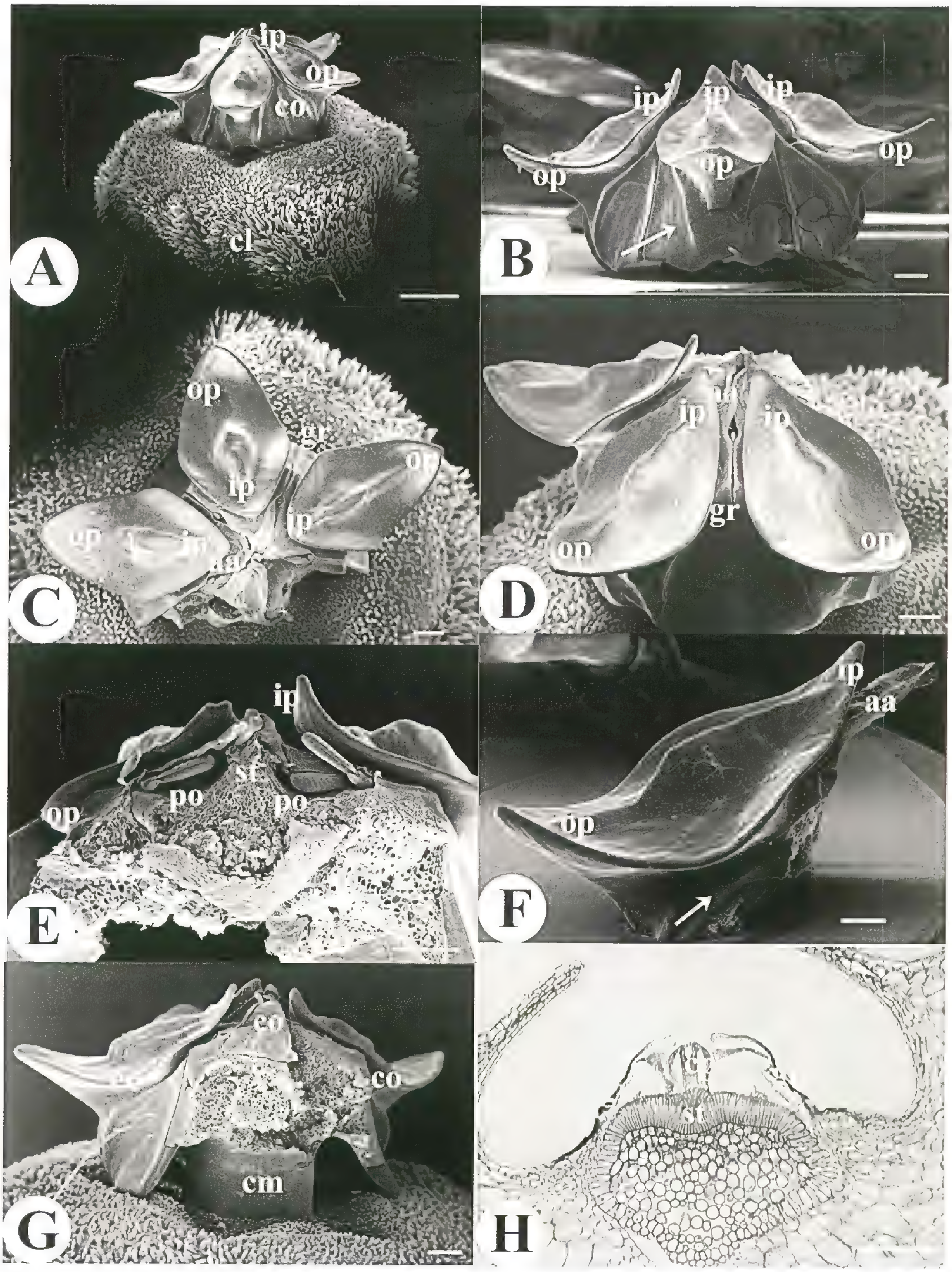

Figure 7. *Hoya lacunosa*. -A. Flower with corolla (cl) and inner (ip) and outer (op) corona (co) processes. —B–D. Different views of coronas with inner (ip) and outer (op) processes, anther appendages (aa), and guide rails (gr). Arrow shows the fused outer corona processes. —E. Section of flower showing pollinia (po) and style head (st). —F. Single corona lobe with inner (ip) and outer (op) processes and adnate anther appendage (aa). Arrow shows the fused outer corona processes. —G. Corona (co) seen from side showing column (cm). —H. Cross section of flower with guide rails (gr), corpusculum (cp), and style head (st). Scale bars = 1 mm (A), 100 µm (H), 200 µm (F), 300 µm (B–E, G).

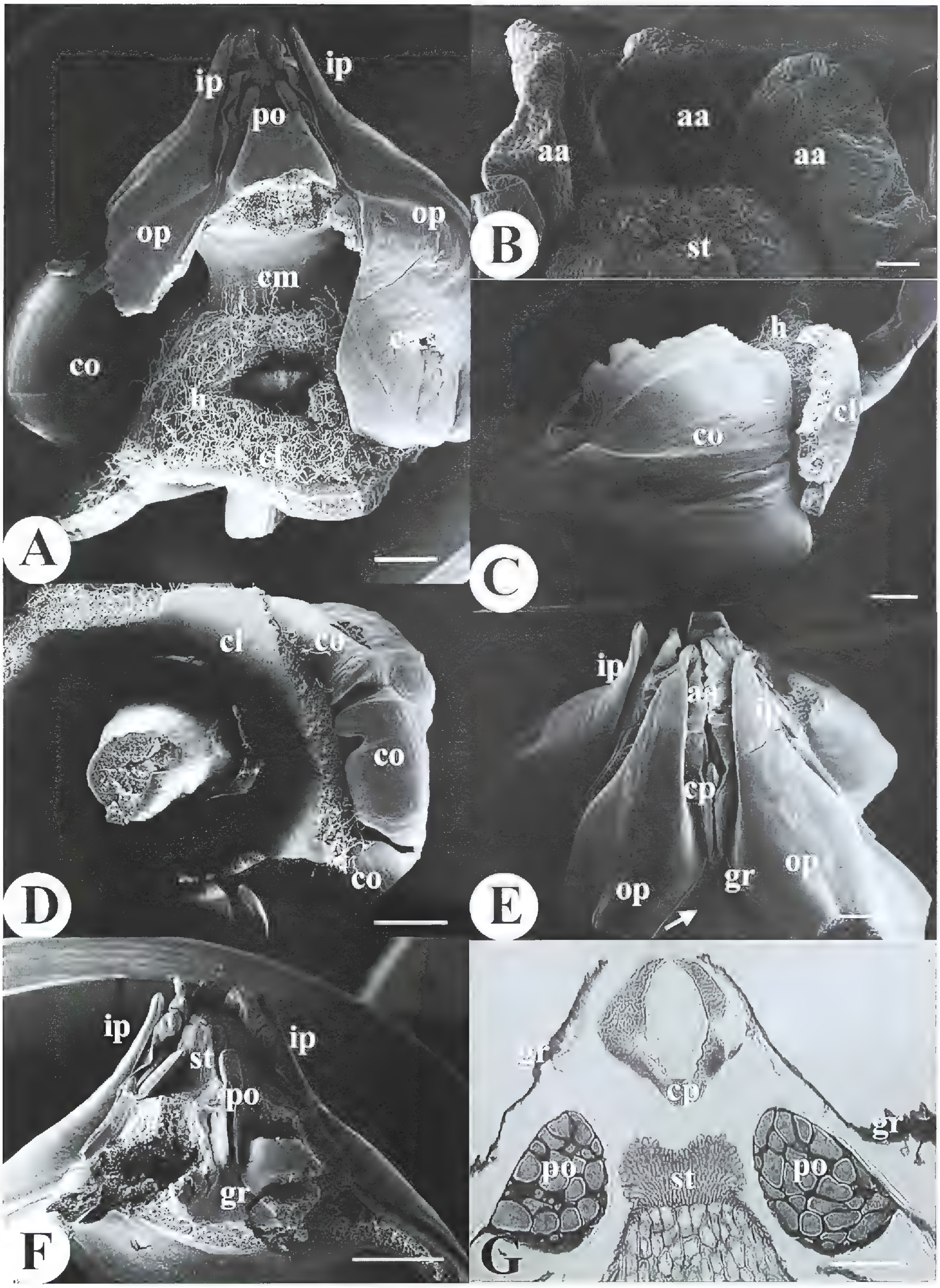

Figure 8. *Hoya inflata*. —A. Flower showing the remainder of the corolla (cl) with hairs (h), corona (co) with inner (ip) and outer (op) corona (co) processes, column (cm), and pollinia (po). —B. Upper part of flowers with anther appendages (aa) and style head (st). —C. Flower seen from side with corona (co) and the rest of corolla (cl) with hairs (h). —D. Flower seen from below with the remainder of the corolla (cl) and corona processes bent inward. —E–G. Upper part of flowers showing inner (ip) and outer (op) corona processes, guide rails (gr), anther appendages (aa), pollinia (po), style head (st), and corpuscula (cp). Arrow in E shows the fused processes of the corona. Scale bars = 1 mm (A, C–F), 100 mm (B, G).

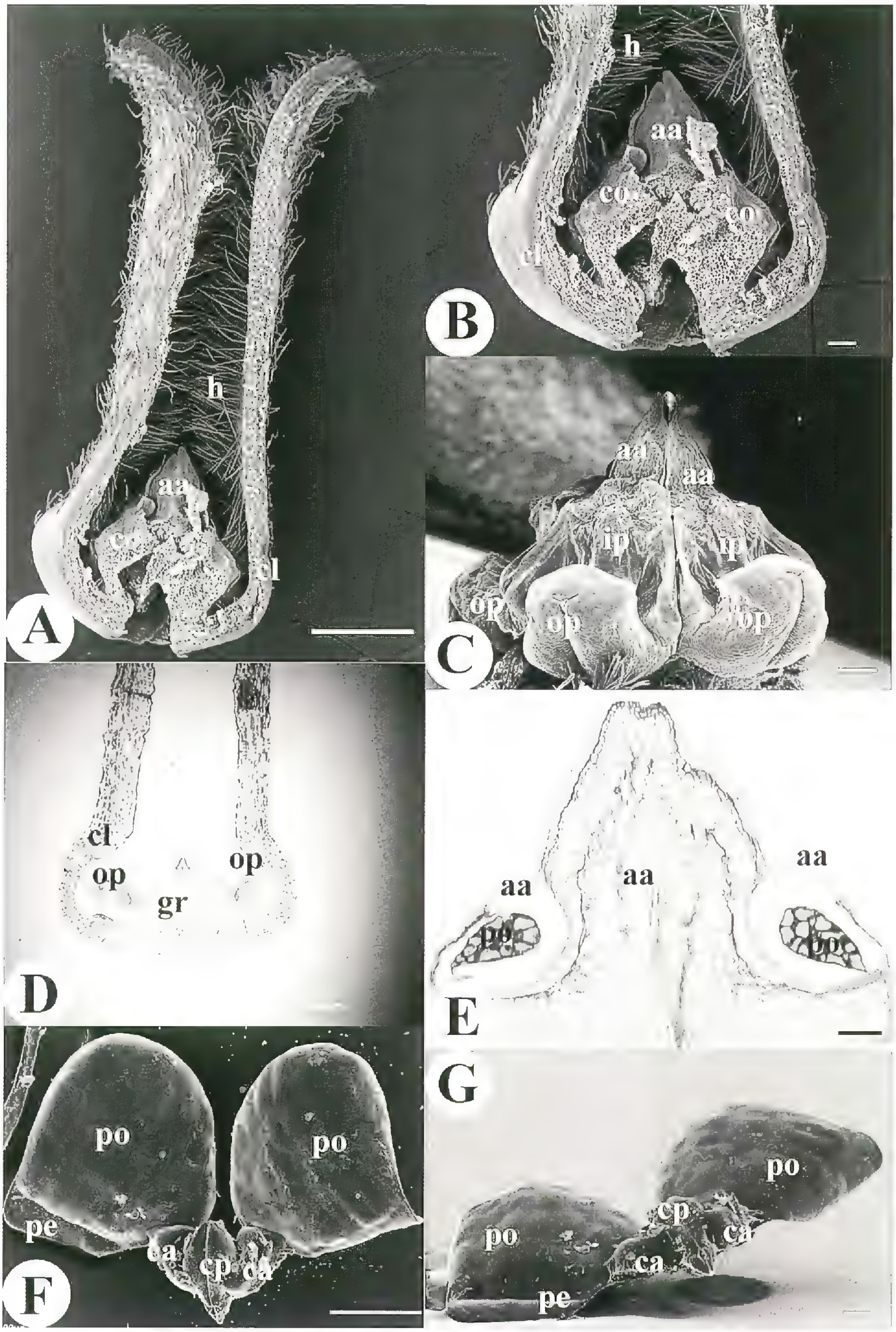

Figure 9. *Hoya yuennanensis.* —A, B, D. Longitudinally sectioned flowers with corolla (cl) with hairs (h), corona (co) with revolute outer processes (op), guide rails (gr), and anther appendages (aa). —C, E. Upper part of flower with inner corona processes (ip), revolute outer processes (op), anther appendages (aa), and pollinia (po). —F, G. Pollinaria with corpusculum (cp), caudicles (ca), and pollinia (po) with pellucid margins (pe). Scale bars — 1 mm (A), 1 mm (D), 30 μm (G), 100 μm (E, F), 200 μm (B, C).

0.1 × 0.08 mm, ending with 2 short and straight appendages (Fig. 9F, G). Caudicles ca. 0.1 × 0.1 mm (Fig. 9F, G).

8. Hoya patella Schltr., Bot. Jahrb. Syst. 50: 132. 1913. TYPE: New Guinea. *Schlechter 16375* (holotype, B not seen).

Hoya patella is an epiphyte with pendent or weakly twining stems and fleshy leaves from New Guinea. Inflorescences pedunculate, comprising racemes with 1 to 5 flowers; pedicels ca. 50 mm long, glabrous. Calyx lobes obtuse-triangular, ca. 2 × ca. 1 mm. Corolla campanulate, light pink, externally glabrous, hirsute, internally with reticulate nervature, ca. 3 cm diam.; lobes triangular-ovate, reflexed, ca. 5 × 2 mm, with hairy margins (Fig. 10A). Gynostegium on a short column (Fig. 10A). Corona much smaller than the corolla. dark red, smooth; inner processes slightly ascending (Fig. 10B), shorter than anther appendages (Fig. 10D, E); outer processes revolute but with margins that do not touch (Fig. 10C, G); anther appendages lanceolate, acute, exceeding the corona lobes (Fig. 10D, E); style head shortly apiculate (Fig. 10D, E, H). Pollinarium comprising 2 pollinia connected by short, flattened, winged caudicles to a corpusculum; pollinia longer than wide, cylindrical-elongate, protruding outward at the basal part, ca. 0.7 × 0.3 mm (Fig. 10F); pellucid margin covering the entire dorsal margin of the pollinia (Fig. 10F); corpusculum rhomboid, ca. 0.2 × 0.1 mm, ending with 2 short appendages (Fig. 10F); caudicles ca. 0.1 × 0.03 mm (Fig. 10F).

9. Hoya retusa Dalzell, Kew J. Bot. 4: 294. 1852. *Hoya retusa* Warb. Repert. Spec. Nov. Regni Veg. 3: 344. 1907, nom. illeg. TYPE: Ind. Or. *Dalzell s.n.* (holotype, K not seen).

Hoya retusa is an epiphyte with non-twining pendent stems and fleshy foliage from India. Inflorescences pedunculate, comprising a much-reduced raceme carrying only a single flower; pedicels ca. 10 mm long. Calyx lobes elliptic-oblong, ca. 0.33 × 0.2 mm. Corolla flattened-campanulate, white, ca. 15 mm diam.; lobes triangular-acute, pubescent, ca. 5 × 2 mm. Corona outer processes keel-shaped, dark red; inner processes acutely ascending, outer processes deeply revolute (Fig. 11A–C, E); anther appendages lanceolate, acute, noticeably exceeding the corona lobes (Fig. 11B, C); style head shortly apiculate (Fig. 11A, D). Pollinarium comprising 2 pollinia connected by broadly flattened, unwinged caudicles to a corpusculum; pollinia oblong-elongate, ca. 0.4 × 0.2 mm (Fig. 11F); corpusculum ca. 0.3 × 0.1 mm (Fig. 11F); caudicles ending with 2 short appendages, ca. 0.04 × 0.02 mm (Fig. 11F).

10. Hoya venusta Schltr., Bot. Jahrb. Syst. 50: 128. 1913. TYPE: New Guinea. *Schlechter 18767* (holotype, B not seen).

Hoya venusta is an epiphytic climber with fleshy leaves from New Guinea. Inflorescences pedunculate, comprising umbels with 10 to 15 flowers, ca. 1.5 cm diam. Calyx lobes obtuse, 1–2.5 × 0.5 mm, glabrous. Corolla campanulate, white to dark pink, outside glabrous, internally papillose-hairy; lobes obovate, 5–7 × 5–6 mm. Gynostegium on a short column (Fig. 12A). Corona creamy white with light red center, striate; inner processes ascending, covering the style head (Fig. 12B–D), central part of the lobes with an umbo (Fig. 12B, F); outer processes deeply revolute (Fig. 12E); anther appendages lanceolate-acute, much exceeding the corona lobes (Fig. 12C, D); style head shortly apiculate (Fig. 12B–D). Pollinarium comprising 2 pollinia connected by broadly flattened, unwinged caudicles to a corpusculum; pollinia obovoid-oblong, ca. 0.4 × 0.2 mm (Fig. 12G, H); corpusculum small, rhomboid, size not measured (Fig. 12G, H); caudicles ca. 0.1 × 0.04 mm (Fig. 12G, H).

Discussion

In his generic revision of the Marsdenieae, Omlor (1998) considered the genera *Absolmsia*, *Dischidia*, *Hoya*, *Madangia*, and *Micholitzia* to form a group (the *Hoya*-group) characterized by flowers having coronas with revolute outer processes, pollinia with pellucid margins, and fusiform seeds without prominent wings. Likewise, a close relationship between the genera *Hoya*, *Dischidia*, and *Micholitzia* has been reported in several molecular phylogenetic studies (e.g., Potgieter & Albert, 2001; Livshultz, 2002, 2003; Rapini et al., 2003; Meve & Liede, 2004). However, none of these studies resolved the phylogenetic relationship between these genera. In order to explore the boundaries of *Hoya*, Wanntorp et al. (2006a, b) analyzed 35 species of this genus and close relatives of the Marsdenieae. The monotypic genera *Absolmsia*, *Madangia*, and *Micholitzia* were found nested within *Hoya* with strong support (Wanntorp et al., 2006a, b).

Below, we discuss the position of *Absolmsia spartioides*, *Madangia inflata*, and *Micholitzia obcordata* nested within *Hoya* by comparing their flower morphology with that of closely related species of *Hoya*.

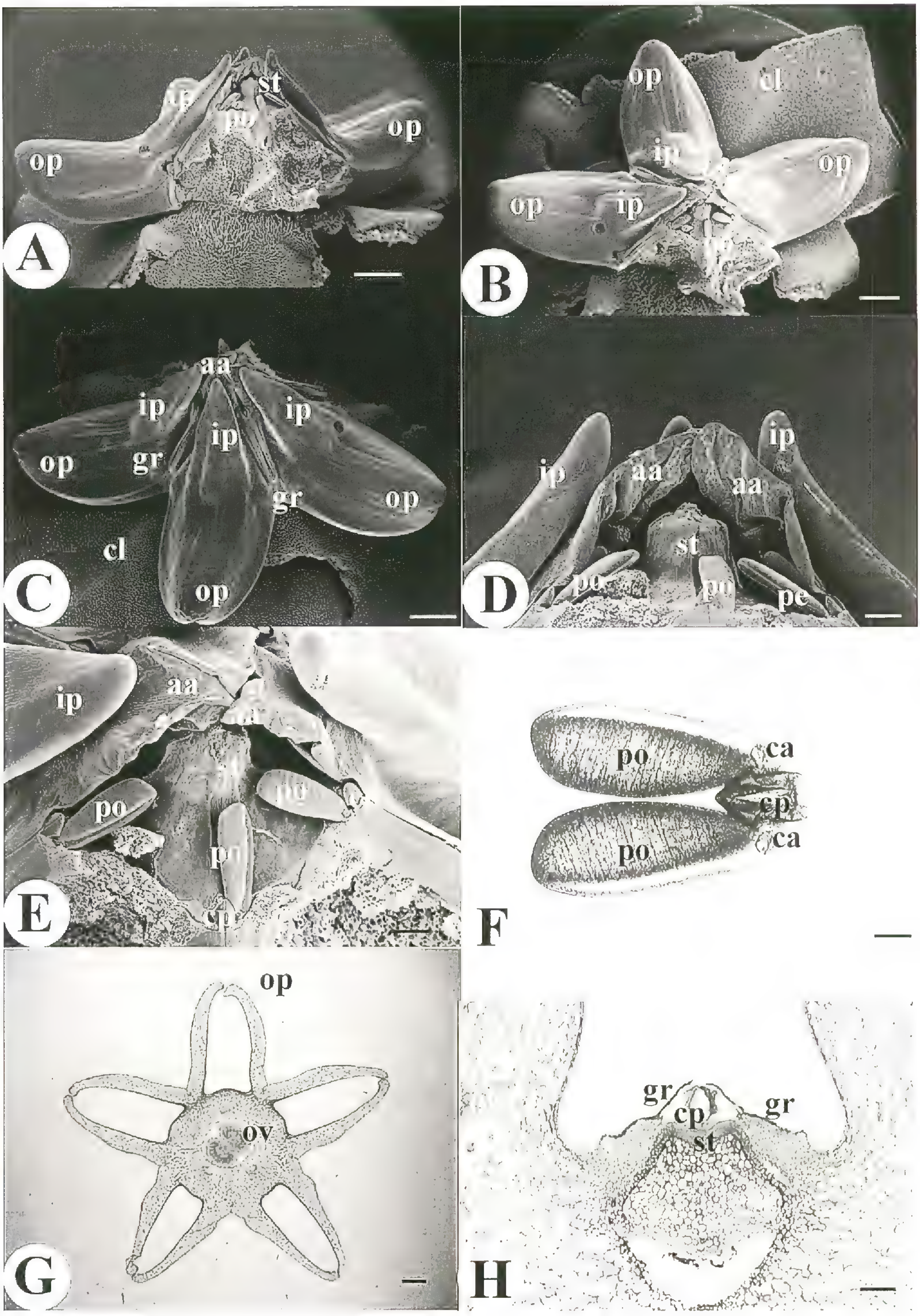

Figure 10. *Hoya patella*. —A–C. Side (A) and above (B, C) views of flowers with corolla (cl), corona with revolute outer processes (op), inner processes (ip), anther appendages (aa), guide rails (gr), pollinia (po), and style head (st). —D, E. Upper part of flower with inner (ip) corona processes, anther appendages (aa), pollinia (po) with pellucid margins (pe), corpusculum (cp), and style head (st). —F. Pollinarium with pollinia (po), corpusculum (cp), and caudicles (ca). G. Cross section of lower part of flower showing ovary (ov) and outer corona (op) processes. —H. Longitudinal section of flower showing position of guide rails (gr), corpuscula (cp), and style head (st). Scale bars = 1 mm (A–C), 1.5 mm (G), 100 µm (F, H), 200 µm (D, E).

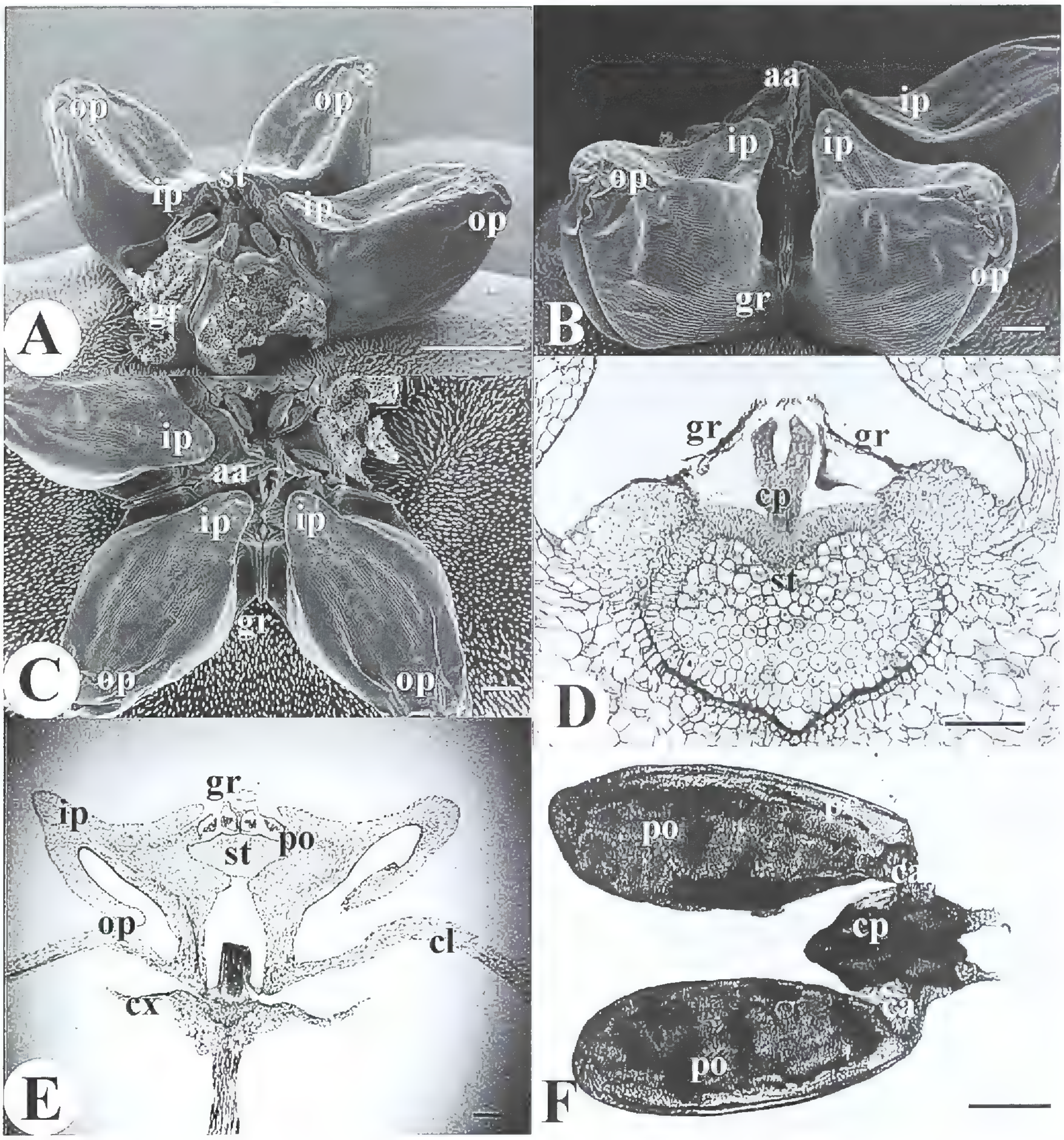

Figure 11. *Hoya retusa*. —A. Sectioned flower showing inner (ip) and outer (op) revolute corona processes, guide rails (gr), and style head (st). —B, C. Corona seen from side (B) and from above (C) showing inner (ip) and outer (op) revolute corona processes, guide rails (gr), and anther appendages (aa). —D. Longitudinal section of upper part of flower with guide rails (gr), corpusculum (cp), and style head (st). —E. Longitudinal section of flower showing calyx (cx), corolla (cl), inner (ip) and outer (op) corona processes, guide rails (gr), style head (st), and pollinia (po). —F. Pollinarium with pollinia (po), pellucid margins (pe), corpusculum (cp), and caudicles (ca). Scale bars = 1 mm (A), 3 mm (B), 100 μm (D, F), 300 μm (C, E).

THE GENERIC STATUS OF *ABSOLMSIA*

At first sight, mature plants of *Absolmsia* look very unlike *Hoya* because they lack the leaves characteristic of that genus. Leaves are only present in very young plants of *Absolmsia*; in mature plants, photosynthesis is instead carried out mainly by long, green, perennial peduncles of the inflorescences. In most species of *Hoya*, the peduncles are also perennial; only a few *Hoya* species have annual peduncles, a derived character state in this genus and almost entirely found in the section *Eriostemma* Schltr.

Absolmsia spartioides was first described by Bentham in 1880 as *Astrostemma spartioides* Benth., but this name was later changed to *Absolmsia* by Kuntze, because he regarded it as too similar to the name of another genus of tribe Marsdenieae, *Asterostemma* Decne. (Omlor, 1998). The similarity between *Absolmsia* and *Hoya* was first pointed out by Schlechter (1916: 2), who stated that "*Absolmsia*, placed by K. Schumann within Cynanchinae, belongs

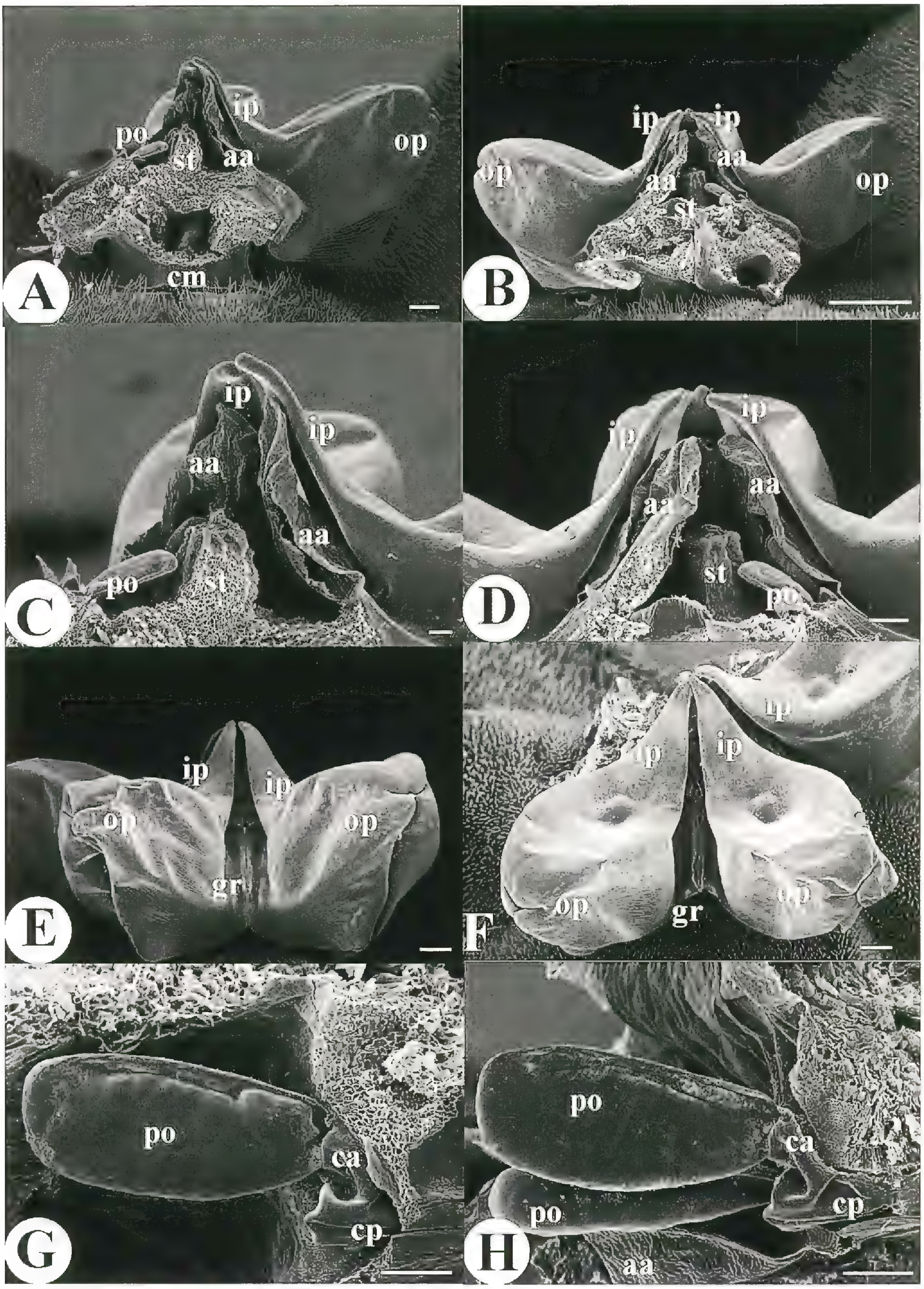

Figure 12. *Hoya venusta*. —A–D. Details of flowers showing the corona with inner (ip) and outer (op) revolute processes, column (cm), anther appendages (aa), style head (st), and pollinia (po). —E, F. Coronas seen from side showing guide rails (gr). —G, H. Pollinaria adnate to anther appendage (aa), with pollinia (po), corpuscula (cp), and caudicles (ca). Scale bars — 1 mm (B), 100 µm (C, G, H), 200 µm (D), 300 µm (A, E, F).

without any doubts near to *Hoya*." Omlor (1998) included *Absolmsia* in a *Hoya*-group based on several morphological characters common to both of these genera: a corona with inner processes that cover the anthers and outer revolute processes (Fig. 1D, E); pollinia with a dorsal pellucid margin (Fig. 2H); and almost round seeds with thickenings on the lateral walls of cells in their seed coats. Based on morphological similarities in the shapes of the corona and corolla, Kloppenburg reduced *Absolmsia* to synonymy under *Hoya*, transferring the name as *H. spartioides* (Kloppenburg, 2001).

The available molecular phylogeny for *Hoya* indicates that the sister group of *Absolmsia spartioides* is *H. curtisii* and that these two species are part of a weakly supported clade also including *H. lacunosa* and a well-supported subclade with *H. imbricata* and *H. caudata* (Wanntorp et al., 2006b). *Absolmsia spartioides* and *H. curtisii* differ substantially in flower morphology, the latter having a corolla with completely reflexed lobes and a corona lying on an annulus (Fig. 4A, B), while *Absolmsia* has a campanulate corolla and a corona without any annular structure (Fig. 1A, C). Because the sample of *Hoya* species included in the molecular studies of Wanntorp et al. (2006a, b) was limited to only 35 out of over 300 species of *Hoya*, it is probable that the retrieved sister relationship between *A. spartioides* and *H. curtisii* will change by the inclusion of additional species of *Hoya* in the molecular phylogeny. Although it is not possible at this stage to point to a particular species of *Hoya* as sister to *Absolmsia*, the inclusion of this genus in *Hoya* was nevertheless clearly shown in the tree of Wanntorp et al. (2006a, b) and is in concordance with previous morphological studies (Omlor, 1998; Kloppenburg, 2001).

The shape of the corolla varies extensively in *Hoya* from rotate with revolute lobes (e.g., *H. lacunosa*, Fig. 7A), flattened-campanulate in *H. caudata* (Fig. 3A), or urceolate to globose in *H. heuschkeliana* Kloppenb. and *H. telosmoides* R. Omlor (Omlor, 1996). The corolla lobes are commonly reflexed as in *H. curtisii*. Corolla characters (especially the shape) in *Hoya* seem highly labile and have undoubtedly evolved numerous times. The shape of the corona, on the other hand, seems to be a character of higher phylogenetic value for linking *Absolmsia* to *Hoya*, as both have coronas with deeply revolute outer processes to the lobes (Omlor, 1998; Fig. 1D, E). In addition, the gynostegium of *Absolmsia*, as in many species of *Hoya*, is raised above the corolla on a column that varies in the degree of indument and in conspicuousness. This column is short in *Absolmsia* (Fig. 1C), *H. patella* (Fig. 10A), and *H. caudata* (Fig. 3B) and not as conspicuous as in *H. curtisii* (Fig. 4D). Pollinia of *Absolmsia*, *Hoya*, *Micholitzia*, and *Madangia* have a pellucid dorsal margin in which pollen tubes germinate (Omlor, 1998; this study). This trait is unknown in the genus *Dischidia* (Meve, 2001) and all other species of the Marsdenieae (Omlor, 1998). These morphological characters and the molecular results (Wanntorp et al., 2006a, b) support the inclusion of *Absolmsia* in *Hoya*, transferred by Kloppenburg (2001) as *H. spartioides* (Benth.) Kloppenb.

THE GENERIC STATUS OF *MADANGIA*

Forster et al. (1997) collected a plant in the Madang province of New Guinea that very much resembled species of *Hoya* in its epiphytic habit and fleshy leaves. The plant was assigned to the monotypic genus *Madangia* on the basis of floral morphological characters unique for this species. One of these characters was the globose shape of the corolla, which is unusual in *Hoya* as discussed by Forster et al. (1997), who mentioned *H. heuschkeliana* as the only species with similar corolla shape. Omlor (1996) also mentioned a species from Sabah, *H. telosmoides*, with a similar corolla shape, although this species has corollas internally covered with long recurved hairs. *Madangia inflata* is well supported as sister species to the New Guinean *H. hypolasia*, both being part of a larger clade, including other species from Australia and New Guinea (e.g., *H. patella*, *H. venusta*, and a subclade with *H. australis* R. Br. ex J. Traill and *H. albiflora* Zipp. ex Blume) (Wanntorp et al., 2006a, b). *Hoya heuschkeliana*, on the other hand, was associated with species from the *Acanthostemma* Blume group, which have pubescent corollas and completely reflexed lobes that give the flowers a globular shape. *Hoya telosmoides* was not found close to *M. inflata* in the tree (Wanntorp et al., 2006b). These results again support the plasticity of the corolla in *Hoya* and underline the hazard of establishing too much reliance upon single corolla characters in the recognition of genera in the group.

The corona of *Madangia inflata* has a remarkable shape, which contributed to the decision of Forster et al. (1997) to establish a new genus for this species. The five inner corona processes are free at the apical part near the style head as in *Hoya*, but their outer parts, corresponding to the outer processes of the corona lobes in *Hoya*, form a continuous rim that is folded inward (Fig. 8D). A similar corona shape, which has not been previously discussed, is found in *H. lacunosa* (Fig. 7B). In this species also, the five outer corona lobes are fused at their basal part, forming a skirt that somewhat resembles the membranous corona of *Madangia* (Fig. 8D). According to

Wanntorp et al. (2006b), *H. lacunosa*, however, groups with many other species of *Hoya* and is clearly separated from *M. inflata*. This corona type therefore seems to have arisen independently several times in *Hoya*, paralleling the variation in corolla morphology.

The remaining flower parts of *Madangia* are very similar to *Hoya*. The column between the corolla and the gynostegium is very conspicuous in *Madangia* as in many species of *Hoya*, such as *H. curtisii*, *H. lacunosa*, and *H. venusta* (Figs. 4D, 7G, 12A). The pollinia of *Madangia* (Fig. 2C, D) have a clear pellucid margin as in *Hoya* (e.g., Fig. 6G). Another interesting character not previously noted in *Madangia* is the dimerous style head that is flat and shortly apiculate in both *Madangia* and *H. hypolasia* (Figs. 8B, 6C) and shortly apiculate-conical in *H. macgillivrayi* F. M. Bailey, *H. venusta* (Fig. 12C, D), and *H. patella* (Fig. 10D) of the same clade. The shape of the style head and the morphology of the pollinaria are in agreement with the molecular results of Wanntorp et al. (2006b), and we propose that *Madangia* should be included in *Hoya* as *H. inflata*.

THE GENERIC STATUS OF *MICHOLITZIA*

During an expedition to India and Burma, the orchid collector W. Micholitz discovered a new taxon, later described as *Micholitzia obcordata* (Brown, 1909). *Micholitzia* was again collected in China by Handel-Mazzetti and in India by Deb and named, respectively, *H. yuennanensis* Hand.-Mazz. and *H. manipurensis* Deb. In 1974, Tsiang and Li collected the same plant in China, thought it to be a new species of *Hoya* that they called *H. lantsangensis* Tsiang & P. T. Li, and erected a new section *Antiostelma* for it. Maxwell transferred *Micholitzia* to *Dischidia* (1991), and P. T. Li (1992: 218) argued that the cylindric shape of the corollas and the bud aestivation, which "twisted leftward," were different enough from those of *Hoya* and *Dischidia*, with valvate aestivation and rotate and urceolate corollas, respectively, to raise section *Antiostelma* in *Hoya* to generic rank. According to molecular evidence (Wanntorp et al., 2006a), *Micholitzia* is nested within *Hoya*, even though its sister relationship to any of the species of *Hoya* is less clear. In habit, *Micholitzia* is more similar to *Absolmsia* than to *Hoya*, *Dischidia*, or *Madangia*. In these last three genera, but not in *Micholitzia* and *Absolmsia*, adventitious roots are produced at the leaf nodes (Goyder & Kent, 1994). The corolla of *Micholitzia* is of a similar urceolate or tubular shape to that of a few species of *Hoya* (e.g., *H. heuschkeliana*) but is wider at the lower part of the corollas (Goyder & Kent, 1994). This shape superficially resembles that of many species of *Dischidia*, but in this genus usually only the upper internal part of the corolla is pubescent, while in *Micholitzia* the corollas are uniformly pubescent on the outside and inside (Goyder & Kent, 1994). The corona of *Micholitzia* has been considered to be different from that of *Dischidia* and *Hoya* because its five fleshy and almost round lobes do not end as in *Hoya* with five pointed inner processes on the anther appendages. Its inner corona processes are instead blunt-ended and appear almost as if they were united to the five conspicuous anther appendages hiding the flat and short style head. Despite the different inner corona processes of *Micholitzia*, the outer processes of the corona lobes have revolute margins (Fig. 9C) similar to those of *Hoya* (e.g., *H. retusa*) (Omlor, 1998; Fig. 11B). In addition, the pollinaria of *Micholitzia* have clear pellucid margins (Omlor, 1998; Fig. 9F). Clear pellucid margins are also present in *Absolmsia* (Fig. 2H), *Madangia* (Fig. 2C), and *Hoya* (e.g., Fig. 5E) but are absent in *Dischidia*. The position of the pellucid margin of *Micholitzia*, however, is superficially different from *Hoya* because it is apparently situated at the lower part of the pollinia instead of at the outer edge (Fig. 9F), but as observed by Goyder and Kent (1994), this is due to the twisting of the pollinia of *Micholitzia*. By rotating the pollinia at 90°, the pellucid margin takes the same position as in the pollinia of *Hoya*. Based on the molecular evidence for *Hoya* and on the similarity of the pollinia and of the outer corona processes, *Micholitzia* should be included in a broadly defined *Hoya*. Due to the prior use of the epithet *obcordata* in *Hoya*, inclusion of *Micholitzia obcordata* in *Hoya* necessitates a change of epithet for the species, based on the next available name of the several synonymous species, which is *H. yuennanensis*.

CONCLUSION

A recurring theme in recent molecular-based phylogenies within the Apocynaceae, Asclepiadoideae is that morphological classifications based on single, albeit unusual, floral features (especially of the corolla or coronas) are not corroborated by molecular data (e.g., Meve & Liede, 2001, 2002, 2004). In the *Hoya* group, the molecular phylogeny to date (Wanntorp et al., 2006b) indicates relationships that follow geographical patterns, as shown by the clade including almost exclusively Australian and New Guinean species, but also partly reflect morphological similarity, as in *Madangia*, *Absolmsia*, and *Micholitzia*.

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PHYLOGENETIC RELATIONSHIPS IN THE TRIBES ANCHONIEAE, CHORISPOREAE, EUCLIDIEAE, AND HESPERIDEAE (BRASSICACEAE) BASED ON NUCLEAR RIBOSOMAL ITS DNA SEQUENCES

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Abstract

Sequence data from the nuclear ITS region of 118 species (152 accessions) were used to test the monophyletic status and interrelationships of four related tribes in the Brassicaceae: Anchonieae, Chorisporeae, Euclidieae, and Hesperideae. Both maximum parsimony and maximum likelihood analyses support the recognition of the tribes Hesperideae (unigeneric, *Hesperis* L.) and Chorisporeae (3 genera, including *Chorispora* R. Br. ex DC., *Diptychocarpus* Trautv., and *Parrya* R. Br.), whereas the Anchonieae and the Euclidieae each separate into two distinct and distant clades (designated here as Anchonieae I and II and Euclidieae I and II). The data also support the exclusion of eight genera from the latter four tribes, with appropriate tribal assignment given in parentheses: *Aubrieta* Adans. (Arabideae), *Blennodia* R. Br. (Camelineae), *Erysimum* L. (Camelineae), *Goldbachia* DC. (unresolved), *Hesperidanthus* (B. L. Rob.) Rydb. (Schizopetaleae), *Votothlaspi* Hook. f. (unresolved), *Pseudocamelina* (Boiss.) N. Busch (Thlaspideae), and *Zuvanda* (Dvorák) Askerova (unresolved). The genus *Malcolmia* R. Br. (ca. 35 species) was paraphyletic and divided into three tribal clades, providing support for the separate genera *Malcolmia*, *Strigosella* Boiss., and *Zuvanda*. The genera *Anchonium* DC., *Desideria* Pamp., *Eremobium* Boiss., *Erysimum*, *Iskandera* N. Busch, *Matthiola* R. Br., *Morettia* DC., *Veotorularia* Hedge & J. Léonard, *Rhammatophyllum* O. E. Schulz, *Sisymbriopsis* Botsch. & Tzvelev, *Sterigmostemum* M. Bieb., and *Tetracme* Bunge each fell within a single tribal clade but were not monophyletic.

Key words: Anchonieae, Chorisporeae, Euclidieae, Hesperideae, ITS, Brassicaceae.

The Brassicaceae are a large family of ca. 338 genera and over 3700 species distributed throughout the world, primarily in temperate regions (Al-Shehbaz, 1984; Al-Shehbaz et al., 2006; Warwick & Al-Shehbaz, 2006). They are a natural family easily distinguished by floral and fruit morphology (e.g., cruciform corolla, tetradynamous stamens, and characteristic siliques). Tribal classification of the family is, however, problematic and not well understood phylogenetically. The characters traditionally used at this rank are few and include orientation of the radicle in the embryo, number of rows of seed in each locule, flower color, trichome type, and features of the nectaries. Numerous studies have shown these morphological characters to be highly homoplasious and therefore not phylogenetically informative on a family-wide basis (reviewed in Al-Shehbaz et al., 2006). Several tribal systems have been proposed and, with the exception of the Brassiceae, few, if any, of the other traditional tribes are monophyletic (Hedge, 1976; Al-Shehbaz, 1984; Koch et al., 2001; Al-Shehbaz et al., 2006).

The first comprehensive molecular-based overviews on the phylogeny of the family are now emerging (Bailey et al., 2006; Beilstein et al., 2006). The present paper tests the monophyletic status and interrelationships of four closely related tribes in the Brassicaceae: the Anchonieae DC. (12 genera), Chorisporeae Ledeb. (2 genera), Euclidieae DC. (25 genera), and Hesperideae Prantl (1 genus), as recently redefined by Al-Shehbaz et al. (2006). In Beilstein et al. (2006), these tribes corresponded to four separate, strongly supported *ndh*F-based clades, designated Q, S, P, and R, respectively, within one of three well-supported lineages, designated III in figure 2 of their study. Sampling from these tribes, however, was limited in that study, with sequence data from only 4, 2, 12, and 2 taxa, respectively. Similarly, few sequences were available for these tribes in the ITS-based study of Bailey et al. (2006), i.e. Anchonieae (2

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Table 1. Comparison of traits in the four related tribes Anchonieae, Chorisporeae, Euclidieae, and Hesperideae, modified from Al-Shehbaz et al. (2006).

Trait	Anchonieae	Chorisporeae	Euclidieae	Hesperideae
Trichomes	branched	simple, not branched	branched and simple	simple and/or forked
Glands	multicellular	multicellular	glands absent	unicellular
Stalk of glands	multiseriate	multiseriate	glands absent	uniseriate
Siliques	dehiscent, not splitting into 1-seeded segments	moniliform fruits, 1-seeded segments	dehiscent or tardily so, no 1-seeded segments	tardily dehiscent
Stigma lobes	strongly 2-lobed	connivent	entire or 2-lobed	decurrent
Sepals	erect	erect, closed calyx	erect	erect
Cotyledons	accumbent or incumbent	accumbent	incumbent	incumbent
Base chromosome number	$x = 7$	$x = 7$	$x = 7$	$x = 6$–10
Number of genera	12	2	25	1
Number of species	130	12	150	46

genera), Hesperideae (1 species), Euclidieae (3 genera); the three tribes formed a loosely supported single clade, with greater support for the Euclidieae (70% bootstrap support). These tribes also formed a clade in recent *trn*F-based sequence studies of Koch et al. (2006), with further support for their relatedness suggested by the shared presence of a large insertion in the *trn*L intron. Similarly, in *mat*K and *Chs*-based sequences studies, five genera assigned to these tribes formed a well-supported monophyletic group (Yue et al., 2006).

All genera placed in the tribes Anchonieae, Chorisporeae, and Hesperideae have glands, whereas members of the Euclidieae do not (Table 1; Al-Shehbaz et al., 2006). These glands are multicellular with multiseriate stalks in the Anchonieae and Chorisporeae, but unicellular on uniseriate stalks in the Hesperideae. The primary difference between the Anchonieae and Chorisporeae is the presence of 1-seeded fruit segments in the latter tribe. Genera in the four tribes have been subjected to various tribal placements in the past (see Table 2). Schulz (1936) placed the genera with multicellular glands, along with a highly heterogeneous assemblage of other genera, in the tribes Hesperideae and Matthioleae O. E. Schulz, distinguishing the two solely on the basis of cotyledonary position, and recognized the Euclidieae as a separate tribe. These three tribes were later combined by Janchen (1942) as the Hesperideae, subtribes Hesperidinae, Matthiolinae, and Euclidiinae, respectively. Al-Shehbaz (1984) recognized a single large tribe, the Hesperideae, which included the Anchonieae, Cheirantheae Webb & Berth., Erysimeae Dumort., Matthioleae, and Schizopetaleae R. Br. ex Barn. Al-Shehbaz (1988) later combined the Hesperideae and Matthioleae under the earlier name Anchonieae (ca. 27 genera with multicellular glands and/or connivent decurrent stigmatic lobes). Although *Erysimum* L. was retained in the Anchonieae in his 1988 treatment, Al-Shehbaz noted that careful evaluation of its tribal position was needed.

The Hesperideae, as redefined by Al-Shehbaz et al. (2006), are unigeneric (*Hesperis* L.) and distinguished from the rest of the family by having glands with uniseriate stalks terminated with a unicellular gland. Unicellular glands are also found in *Descurainia* Webb & Berthel., but they are unstalked and apparently not homologous to those of the Hesperideae or the multicellular glands of the tribes Anchonieae and Chorisporeae (Al-Shehbaz, 1988; Al-Shehbaz et al., 2006). The absence of multicellular glands is evident among some members of the Anchonieae and is probably a derived state (Al-Shehbaz, 1988; Jacquemoud, 1988). The genera *Dontostemon* Andrz. ex C. A. Mey., *Hesperis*, *Matthiola* R. Br., and *Parrya* R. Br. include species with and without multicellular glands, while *M. longipetala* (Vent.) DC. and *P. nudicaulis* (L.) Regel often have glandular or eglandular plants within the same population.

The Anchonieae, as defined by Al-Shehbaz et al. (2006), include 12 genera and about 130 species (see Table 2). The *ndh*F-based phylogenetic study of Beilstein et al. (2006) revealed a well-supported Anchonieae clade that included *Matthiola* (2 species), *Oreoloma* Botsch. (1 species), and *Sterigmostemum* M. Bieb. (1 species). The tribe is characterized by the presence of multicellular-multiseriate glands (rarely eglandular), branched trichomes, often strongly 2-lobed stigmas, and erect sepals (Table 1). The tribe is distributed primarily in Eurasia and northern and tropical Africa, with only four species of *Parrya* extending into North America. Dvorák (1972) considered Pacific North America and northeastern Asia as

Table 2. Taxonomic tribal assignment of genera in the Anchonieae, Chorisporeae, Hesperideae, and Euclidieae. Species numbers from Warwick et al. (2006a), and chromosome numbers from Warwick and Al-Shehbaz (2006). Tribal assignments in the present study in conflict with Al-Shehbaz et al. (2006) are indicated in bold. n = haploid chromosome number; $2n$ = diploid chromosome number.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n; $2n$)
Anastatica L.	Euclidieae	Hesperideae-Euclidiinae	—	Euclidieae	—	1	$x = 11$	$2n = 22$
Anchonium DC.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Anchonieae	Anchonieae I	2	$x = 7, 8$	$n = 7$; $2n = 14, 16, 21$
Atelanthera Hook. f. & Thomson	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	—	1	—	—
Aubrieta Adans.	Matthioleae	Arabideae-Arabinae	—	Arabideae	Arabideae	12	$x = 8$	$n = 8$; $2n = 16, 32$
Blennodia R. Br.	Hesperideae	Hesperideae-Hesperidinae	—	—	Camelineae	2	$x = 12$	$n = 12$
Boreava Jaub. & Spach	Euclidieae	Sisymbrieae-Buniadinae	—	Isatideae	—	2	$x = 7$	$2n = 14$
Braya Sternb. & Hoppe	Sisymbrieae-Brayinae	Sisymbrieae-Brayinae	—	Euclidieae	Euclidieae I	13	$x = 14, 16$	$n = 14, 16, 21, 28, 32, 35$; $2n = 28, 32, 42, 48, 56, 64, 70$
Bunias L.	Euclidieae	Sisymbrieae-Buniadinae	Anchonieae	Anchonieae	—	3	$x = 7$	$n = 7$; $2n = 14, 28, 42$
Cheiranthus L. [= *Erysimum* L.]	Hesperideae	Hesperideae-Matthiolinae	—	Camelineae, *Cheiranthus* [= *Erysimum*]	Camelineae			
Chorispora R. Br. ex DC.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Chorisporeae	Chorisporeae	11	$x = 7, 9$	$n = 7, 9, 14$; $2n = 14$
Cithareloma Bunge	Matthioleae	Hesperideae-Matthiolinae	—	Euclidieae	**Euclidieae II**	3	$x = 13$	$n = 13$; $2n = 26$
Clausia Korn.-Trotzky	Hesperideae, *Clausia* [= *Hesperis* L.]	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	**Anchonieae II**	6	$x = 7$	$2n = 14, 28$
Cryptospora Kar. & Kir.	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae I	3	$x = 7$	$2n = 14$
Desideria Pamp.	Arabideae, *Desideria* [= *Ermania* Cham.]	—	—	Euclidieae	Euclidieae I	12	$x = 7$	$2n = 14$
Diceratella Boiss.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Euclidieae	**Euclidieae II**	11	$x = 11$	$n = 11$

Table 2. Continued.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n; $2n$)
Dichasianthus Ovcz. & Junussov	—	—	—	Euclidieae	Euclidieae I	1	—	—
Dilophia Thomson	Lepidieae-Cochleariinae	—	—	Euclidieae	—	2	—	—
Diptychocarpus Trautv.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Chorisporeae	Chorisporeae	1	$x = 7$	$n = 7$; $2n = 14$
Dontostemon Andrz. ex C. A. Mey.	Arabideae	—	Anchonieae	Anchonieae	**Anchonieae II**	11	$x = 7$	$n = 7$; $2n = 14, 28$
Eremobium Boiss.	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	**Euclidieae II**	2	$x = 8, 10, 13$	$n = 10, 13$; $2n = 16, 20$
Erysimum L.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Camelineae	Camelineae	223	$x = 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17$	$n = 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 24, 27, 28$; $2n = 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 48, 54, 56, 72$
Euclidium R. Br.	Euclidieae	Hesperideae-Euclidiinae	—	Euclidieae	Euclidieae I	1	$x = 7$	$n = 7$; $2n = 14$
Goldbachia DC.	Hesperideae	Sisymbrieae-Buniadinae	—	—	**Unresolved**	5	$x = 14$	$n = 14$; $2n = 14, 28$
Hesperidanthus (B. L. Rob.) Rydb.	Matthioleae	Hesperideae-Matthiolinae	—	Schizopetaleae	Schizopetaleae	5	$x = 11$	$n = 11, 20$
Hesperis L.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Hesperideae	Hesperideae	46	$x = 6, 7, 8, 10$	$n = 6, 7, 8, 12, 14, 16, 20, 24$; $2n = 12, 14, 16, 24, 28$
Hymenophysa C. A. Mey. [= *Lepidium* L.]	Euclidieae	Lepidieae-Lepidiinae, *Hymenophysa* [= *Cardaria* Desv.]	Lepidieae	Lepidieae, *Hymenophysa* [= *Lepidium*]	—	—	—	—
Iodanthus Torr. & A. Gray ex Steud.	Matthioleae	Hesperideae-Matthiolinae	Arabideae	Cardamineae	—	1	—	—
Iskandera N. Busch	—	—	Anchonieae	Anchonieae	Anchonieae I	2	—	—
Lachnoloma Bunge	Euclidieae	Hesperideae-Euclidiinae	—	—	—	1	$x = 7$	$2n = 14$

Table 2. Continued.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n; $2n$)
Leiospora (C. A. Mey.) F. Dvorák	—	—	—	Euclidieae	Euclidieae I	6	$x = 7$	$2n = 14$
Leptaleum DC.	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae I	1	$x = 7$	$n = 7$; $2n = 14$
Litwinowia Woronow	Euclidieae, *Litwinowia* [= *Euclidium*]	Hesperideae-Euclidiinae	Anchonieae	—	—	1	$x = 7$	$n = 7$; $2n = 14$
Lonchophora Durieu [= *Matthiola* R. Br.]	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	Anchonieae I	—	—	—
Malcolmia R. Br.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Euclidieae	**Euclidieae I and II; Camelineae**	32	$x = 7, 8, 10$	$n = 7, 8, 10, 14, 16$; $2n = 14, 16, 20, 24, 28, 32$
Maresia Pomel	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	**Euclidieae II**	3	$x = 14$	$2n = 28$
Mathewsia Hook. & Arn.	Hesperideae	Hesperideae-Hesperidinae	—	Schizopetaleae	**Unresolved**	10	—	—
Matthiola R. Br.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	Anchonieae I	48	$x = 5, 6, 7, 8, 13$	$n = 6, 7, 8, 13$; $2n = 10, 11, 12, 14, 16, 24, 28$
Microstigma Trautv.	Matthioleae, *Microstigma* [= *Matthiola*]	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	Anchonieae I	3	$x = 6$	$2n = 12$
Morettia DC.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Euclidieae	**Euclidieae II**	3	$x = 11$	$n = 11$
Myagrum L.	Euclidieae	Sisymbrieae-Isatidinae	—	Isatideae	—	1	$x = 7$	$n = 7$; $2n = 14$
Neotorularia Hedge & J. Léonard	Sisymbrieae-Brayinae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae I	11	$x = 7$	$n = 7$; $2n = 14, 28, 42$
Neslia Desv.	Euclidieae	Lepidieae-Capsellinae	—	Camelineae	Camelineae	1	$x = 7$	$n = 7, 14, 21$; $2n = 14, 42$
Notoceras R. Br.	Matthioleae	Hesperideae-Matthiolinae	—	Euclidieae	**Euclidieae II**	1	$x = 11$	$n = 11$; $2n = 22$
Notothlaspi Hook. f.	Lepidieae-Notothlaspidinae	Lepidieae-Notothlaspidinae	—	Euclidieae	**Notothlaspi clade**	2	$x = 19$	$n = 19$
Ochthodium DC.	Euclidieae	Sisymbrieae-Buniadinae	—	—	—	1	—	—

Table 2. Continued.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n; $2n$)
Octoceras Bunge	Euclidieae	Hesperideae-Euclidiinae	—	—	—	1	$x = 7$	$2n = 14$
Oreoloma Botsch.	—	—	Anchonieae	Anchonieae	Anchonieae I	3	—	—
Parolinia Webb	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Euclidieae	**Euclidieae II**	5	$x = 11$	$2n = 22$
Parrya R. Br.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	**Chorisporeae**	34	$x = 7$	$2n = 14, 21, 28$
Pseudocamelina (Boiss.) N. Busch	Matthioleae	Lepidieae-Cochleariinae	—	Thlaspideae	Thlaspideae	3	$x = 7$	$n = 7$; $2n = 14$
Pseudoclausia Popov	—	—	Anchonieae	Anchonieae	—	10	$x = 7$	$n = 7$; $2n = 14$
Pycnoplinthus O. E. Schulz	Hesperideae	Hesperideae-Hesperidinae	—	—	—	1	—	—
Rhammatophyllum O. E. Schulz	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae I	10	$x = 8$	$2n = 16$
Schimpera Hochst. & Steud. ex Endl.	Euclidieae	Sisymbrieae-Buniadinae	—	Isatideae	—	1	$x = 7$	$n = 7$; $2n = 14$
Shangrilaia Al-Shehbaz, J. P. Yue & H. Sun	—	—	—	Euclidieae	—	1	—	—
Sisymbriopsis Botsch. & Tzvelev	—	—	—	Euclidieae	Euclidieae I	5	—	—
Solms-laubachia Muschl.	Matthioleae	Hesperideae-Matthiolinae	—	Euclidieae	Euclidieae I	9	$x = 7$	$2n = 14, 28$
Spirorhynchus Kar. & Kir.	Euclidieae	Sisymbrieae-Buniadinae	—	Isatideae	—	1	$x = 7$	$2n = 14$
Sterigmostemum M. Bieb.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Anchonieae	Anchonieae I	7	$x = 7$	$n = 7$; $2n = 14$
Strigosella Boiss.	Hesperideae, *Strigosella* [= *Malcolmia*]	Hesperideae-Hesperidinae	Anchonieae, *Strigosella* [= *Malcolmia*]	Euclidieae, *Strigosella* [= *Malcolmia*]	Euclidieae I	4	—	—
Synstemon Botsch.	—	—	—	—	Anchonieae I	2	—	—
Syrenia Andrz. ex Besser	Hesperideae	Hesperideae-Hesperidinae	—	Camelineae, *Syrenia* [= *Erysimum*]	Camelineae	—	—	—
Tauscheria Fisch. ex DC.	Euclidieae	Sisymbrieae-Isatidinae	—	Isatideae	—	1	$x = 7$	$n = 7$; $2n = 12, 14$

Table 2. Continued.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n; 2n)
Tetracme Bunge	Matthioleae	Hesperideae-Matthiolinae	—	Euclidieae	Euclidieae 1	10	$x = 14$	$n = 14$; $2n = 14$, 28
Texiera Jaub. & Spach	Euclidieae	Sisymbrieae-Isatidinae	—	Isatideae, *Texiera* [= *Glastaria* Boiss.]	—	—	—	—
Thelypodiopsis Rydb.	Hesperideae	Sisymbrieae-Thelypodiinae	Thelypodieae	Schizopetaleae	Schizopetaleae	17	$x = 10, 11$	$n = 10, 11$
Thelypodium Endl.	Hesperideae	Sisymbrieae-Thelypodiinae	Thelypodieae	Schizopetaleae	Schizopetaleae	19	$x = 13$	$n = 13$
Veselskya Opiz	Matthioleae, *Veselskya* [= *Pyramidium* Boiss.]	Hesperideae-Matthiolinae	Anchonieae	Euclidieae	—	1	—	—
Zerdana Boiss.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Anchonieae	Anchonieae 1	1	$x = 7$	$2n = 14$
Zuvanda (Dvorák) Askerova	—	—	—	**Unresolved**	—	3	—	—

one evolutionary center for the tribe (as Hesperideae) and central Asia as another. In contrast, Al-Shehbaz (1988) suggested that the occurrence of *Parrya* in the New World more likely represents a recent migration event given that all related genera occur in Central Asia and the greatest diversity of the genus occurs in that part of the world.

The tribe Chorisporeae is exclusively Asian and includes *Chorispora* R. Br. ex DC. and *Diptychocarpus* Trautv. The tribe is characterized by the presence of multicellular-multiseriate glands, the lack of branched trichomes and the presence of connivent stigmas, moniliform fruits breaking into 1-seeded, corky segments, and erect sepals forming a closed calyx (Table 1). Hayek (1911) and Schulz (1936) had also associated *Chorispora* with *Diptychocarpus*, whereas Dvorák (1972) had suggested that *Chorispora* and *Sterigmostemum* were closely related and derived from a common ancestor. Considering the indument and floral morphology, Jacquemoud (1988: 145) suggested that *Chorispora* was instead close to *Hesperis*. In the *ndh*F-based phylogenetic study of Beilstein et al. (2006), *Chorispora* (1 species) and *Diptychocarpus* (1 species) formed a well-supported clade.

As delimited by Al-Shehbaz et al. (2006), the tribe Euclidieae includes about 25 genera and some 150 species. It is primarily Eurasian and north and east African, with only seven species of *Braya* Sternb. & Hoppe found in North America. The tribe is characterized by the lack of multicellular glands and the presence of branched trichomes (rarely simple or absent), and entire or 2-lobed stigmas (Table 1). The *ndh*F-based phylogenetic study of Beilstein et al. (2006) supported a Euclidieae clade with two well-resolved subclades. The first included *Braya*, *Dilophia* Thomson, *Christolea* Cambess., and *Shangrilaia* Al-Shehbaz, J. P. Yue & H. Sun, and the second subclade included *Desideria* Pamp., *Euclidium* R. Br., *Malcolmia* R. Br., *Neotorularia* Hedge & J. Léonard, *Rhammatophyllum* O. E. Schulz, *Sisymbriopsis* Botsch. & Tzvelev, *Solms-laubachia* Muschl., and *Tetracme* Bunge.

The primary objective of this paper was to determine the tribal limits, monophyletic status, and phylogenetic intra-tribal relationships of genera within the related tribes Anchonieae, Chorisporeae, Euclidieae, and Hesperideae as currently defined by Al-Shehbaz et al. (2006) based on a more comprehensive sampling of genera and species than has been published to date. The monophyletic status of genera was also evaluated where multiple congeneric species sampling allowed. Sequences of the internal transcribed spacers of nuclear ribosomal DNA and the 5.8S rRNA gene (collectively, ITS) from a broad analysis of 118 species were used to assess relationships. The ITS region is

currently the most widely used marker in phylogenetic analyses of the Brassicaceae (Koch, 2003; Koch et al., 2003; Warwick et al., 2002, 2004a, 2004b). and a comprehensive, global ITS-based phylogeny for the majority of Brassicaeae species has recently been published (Bailey et al., 2006).

Materials and Methods

Plant Material

Collection data, taxa, voucher numbers, and Genbank accession numbers are listed in Table 3. Ingroup species (101 species, 131 accessions) were selected based on available material and published sequences to represent the four tribes under study: Anchonieae, Chorisporeae, Euclidieae, and Hesperideae. Following the tribal delimitations outlined in Al-Shehbaz et al. (2006), 10 of the 12 genera assigned to the Anchonieae, 19 of the 25 genera assigned to the Euclidieae, and all genera assigned to the Chorisporeae (2 genera) and Hesperideae (1 genus) were included in the present study. Also included were representatives from seven other tribes, defined in Al-Shehbaz et al. (2006), to which previous members of the Anchonieae have been reassigned. These included 17 species (21 accessions) in the Arabideae, Camelineae, Cardamineae, Cochlearieae, Schizopetaleae, Sisymbrieae, and Thlaspideae. Although only poor resolution of the backbone phylogeny of the family Brassicaceae has been obtained with analyses of both ITS (Bailey et al., 2006) and *ndh*F data (Beilstein et al., 2006), these studies have supported the recognition of monophyletic clades corresponding to tribal groupings. Each of the latter seven tribes was supported as a monophyletic clade in previous ITS (Bailey et al., 2006) and *ndh*F studies (Beilstein et al., 2006). Multiple accessions were available for 29 of the species studied (Table 3). Generic types are indicated by an asterisk besides species name. The total ingroup included 118 species and 152 accessions; samples from 73 species (95 accessions) were sequenced in this study and the remainder were obtained from Genbank (Table 3). *Aethionema arabicum* (L.) Andrz. ex O. E. Schulz in Engler & Prantl was selected as the outgroup on the basis of previous molecular phylogenetic analyses, indicating that Aethionemeae Al-Shehbaz, Beilstein & E. A. Kellogg are the most basal tribe in the family (Al-Shehbaz et al., 2006; Bailey et al., 2006; Beilstein et al., 2006; Koch, 2003; Koch et al., 2007).

DNA Extraction, Amplification, and Sequencing

DNA was extracted from approximately 100 mg or less of dried leaf tissue using Bio 101 FastDNA Kit (Qbiogene, Carlsbad, California, U.S.A.) following manufacturer's instructions. All polymerase chain reaction (PCR) amplifications were performed using Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Piscataway, New Jersey, U.S.A.) and approximately 50 ng of genomic DNA in a total of 25 µL on a Thermolyne Amplitron thermocycler (Techne, Princeton, New Jersey, U.S.A.). The entire ITS region (including ITS-1 and ITS-2 of nuclear ribosomal DNA and the 5.8S rRNA gene) was amplified and sequenced as a single fragment using the primers ITS-1 18S described by O'Kane et al. (1997) and ITS-4 (White et al., 1990). The PCR amplification, purification, and sequencing followed Warwick et al. (2004a, b). No cloning of the ITS region was done, as there was no evidence of the presence of two or more ITS sequences in the same individual, as seen for example in *Braya* (Warwick et al., 2004a), where nucleotide additivity or the presence of more than one peak was observed at a particular position in a sequence.

Data Scoring and Analysis

The sequences were automatically assembled using the software ASSEMBLYLIGN (Kodak, Newhaven, Connecticut, U.S.A.), aligned using the Clustal method in MEGALIGN (DNA Star, Madison, Wisconsin, U.S.A.), and further aligned by eye. Sequences of ITS are deposited in Genbank (Table 3), and the full alignment of the ITS data set is available in TreeBASE (www.treebase.org; study accession number SN2686, Pin No. 15130). Boundaries of the ITS-1 and ITS-2 regions were determined by comparisons with the published sequences of *Arabidopsis thaliana* (L.) Heynh. (O'Kane et al., 1997) and *Sisymbrium irio* L. (Warwick et al., 2002). Insertions and deletions (indels) in the ITS region were treated as missing data to retain phylogenetic information from taxa not having the deletion.

Maximum parsimony analyses of 148 (5 of the 152 sequences in the ingroup were identical to that of other accessions as indicated in Fig. 1, and there was one outgroup accession) aligned ITS sequences were conducted using the computer program PAUP*. version 4.0b10 (Swofford, 2002). The most parsimonious trees were generated using the heuristic search algorithm, with tree bisection-reconnection (TBR) branch-swapping, equal-weighted characters, with gaps treated as missing data, 100 random additions of the sampled taxa, and 100 trees saved per replicate. A second heuristic search was conducted using the 50% majority-rule tree obtained in the above analysis as the starting tree and the replicate allowed to swap to completion using the TBR option, with maximum tree number set at 40,000. Statistics relating to the

Table 3. Taxa sequenced for ITS, with collector and collection number for each voucher specimen and geographical origin of the specimen. The taxonomy followed the most recent family-wide Brassicaceae species checklist (Warwick et al., 2006a).

Taxon*	*Collector and No.* (Herbarium[a]); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
1. *Anchonium billardieri* DC.*	*Mouterden 10791* (G)	Lebanon	DQ357512
2. *Anchonium billardieri*	*Reese s.n.*, 18 May 1933 (G)	Lebanon	DQ357513
3. *Anchonium elichrysifolium* subsp. *canescens* (Hausskn. & Bornm. ex Bornm.) Coode & Cullen	*Sorger 69–53–7* (G)	Turkey	DQ357514
4. *Anchonium elichrysifolium* subsp. *elichrysifolium* (DC.) Boiss.	*Davis 15976* (G)	Turkey	DQ357515
5. *Anchonium elichrysifolium* subsp. *elichrysifolium*	*Davis & Hedge 31143* (G)	Turkey	DQ357516
6. *Anchonium elichrysifolium* subsp. *villosum* Coode & Cullen	*Davis & Polunin 23460* (G)	Turkey	DQ357517
7. *Aubrieta deltoidea* (L.) DC.*	Koch et al., 1999a	—	AJ232909‡
8. *Aubrieta parviflora* Boiss.	*Al-Shehbaz et al. 7535* (MO)	Iraq	DQ357518
9. *Blennodia pterosperma* (J. M. Black) J. M. Black	*Weber 9251* (MO)	Australia	DQ357519
10. *Braya humilis* (C. A. Mey.) B. L. Rob.	Warwick et al., 2004a	Canada (N.W.T.)	AY353111‡
11. *Braya humilis*	Warwick et al., 2004a	China	AY353123‡
12. *Chorispora bungeana* Fisch. & C. A. Mey.	*Al-Shehbaz 9237* (MO)	Kazakhstan	DQ357520
13. *Chorispora bungeana*	*Gibbons 0622* (MO)	Afghanistan	DQ357521
14. *Chorispora macropoda* Trautv.	*Gibbons 0407* (MO)	Afghanistan	DQ357522
15. *Chorispora sibirica* (L.) DC.	*Skvortsov s.n.*, 14 July 1989 (MO)	U.S.S.R. (Altai)	DQ357523
16. *Chorispora sibirica*	*Al-Shehbaz 9469* (MO)	Kazakhstan	DQ357524
17. *Chorispora tashkorganica* Al-Shehbaz, T.Y. Cheo, L. L. Lu & G. Yang	*Bartholomew et al. 8369* (MO)	China	DQ357525
18. *Chorispora tenella* (Pall.) DC.*	*Henderson 94–190* (MO)	U.S.A. (Colorado)	DQ357526
19. *Chorispora tenella*	*Kent 11* (MO)	U.S.A. (Colorado)	DQ357527
20. *Cithareloma lehmannii* Bunge*	*Antonio 207* (MO)	Iran	DQ357528
21. *Clausia aprica* (Stephan) Korn.-Trotzky*	*Schischkin s.n.*, 1 June 1931 (LE)	Kazakhstan	DQ357529
22. *Clausia aprica*	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 10099	Mongolia	AY558938.‡ AY558966‡
23. *Clausia aprica*	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 10366	Mongolia	AY558937.‡ AY558965‡
24. *Clausia kasakhorum* Pavlov	*Mechankova 380* (LE)	Kazakhstan	DQ357530
25. *Cryptospora falcata* Kar. & Kir.*	*Rechinger 55502* (G)	Iran	DQ357531
26. *Cryptospora falcata*	*Podlech 21401* (G)	Afghanistan	DQ357532
27. *Desideria incana* (Ovcz.) Al-Shehbaz	Mummenhoff & Muehlhausen, unpubl. Isolate 1031	South Africa	AJ628335.‡ AJ628336‡
28. *Desideria prolifera* (Maxim.) Al-Shehbaz	Mummenhoff & Muehlhausen, unpubl. Isolate 1033	South Africa	AJ628333.‡ AJ628334‡
29. *Diceratella inermis* Jonsell	*Gilbert & Thulin 1573* (MO)	Kenya	DQ357533
30. *Dichasianthus subtilissimus* (M. Pop.) Ovcz. & Junussov*	Warwick et al., 2004a	Tajikistan	AY353169‡
31. *Diptychocarpus strictus* (Fisch. ex M. Bieb.) Trautv.*	*Podlech & Jarmal 30135* (G)	Afghanistan	DQ357534
32. *Diptychocarpus strictus*	*Anders 5502* (G)	Afghanistan	DQ357535
33. *Dontostemon elegans* Maxim.	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 14740	Mongolia	AY558928.‡ AY558956‡
34. *Dontostemon integrifolius* (L.) Ledeb.*	*Ikonnikov Galitzky 1289* (LE)	Mongolia	DQ357536
35. *Dontostemon integrifolius*	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 10217	Mongolia	AY558932.‡ AY558960‡
36. *Dontostemon integrifolius*	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 12308	Mongolia	AY558926.‡ AY558954‡

Table 3. Continued.

Taxon*	*Collector and No.* (Herbarium[a]); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
37. *Eremobium aegyptiacum* (Spreng.) Asch.* in Boiss	*Charpin 24682* (G)	United Arab Emir.	DQ357537
38. *Eremobium longisiliquum* (Coss.) Boiss.	*Podlech 35381* (G)	Algeria	DQ357538
39. *Erysimum asperum* (Nutt.) DC.	Roy (2001)	—	AF183098‡
40. *Erysimum canum* (Pill. & Mitterp.) Polatschek [= *Syrenia cana* (Piller & Mitterp.) Neilr.]	*Bano s.n.*, 21 June 1946 (DAO)	Hungary	DQ357539
41. *Erysimum capitatum* (Douglas ex Hook.) Greene	*Davis 1006* (MO)	U.S.A. (Arizona)	DQ357540
42. *Erysimum capitatum*	Hong et al., 2003	—	AY254534‡
43. *Erysimum cheiri* (L.) Crantz	Hong et al., 2003	—	AY254538‡
44. *Erysimum majellense* Polatschek	Heenan et al., 2002	—	AY028602‡
45. *Erysimum repandum* L.	*Yatskievych & Yatskievych 91 81* (MO)	U.S.A. (Missouri)	DQ357541
46. *Erysimum scoparium* Wettst.	Heenan et al., 2002	—	AY028604‡
47. *Erysimum sisymbrioides* C. A. Mey.	*Al-Shehbaz et al. 9459* (MO)	Kazakhstan	DQ357542
48. *Erysimum witmannii* Zaw.	Francisco-Ortega et al., 1999	Romania	AF040000,‡ AF040043‡
49. *Euclidium syriacum* (L.) R. Br. in W. T. Aiton*	*Mariana Cirta s.n.*, 24 May 1973 (DAO)	Romania	DQ357543
50. *Euclidium syriacum*	*McCaskill 712* (DAO)	U.S.A. (California)	DQ357544
51. *Goldbachia laevigata* (M. Bieb.) DC.*	*Rechinger 51383* (G)	Iran	DQ357545
52. *Goldbachia laevigata*	*Rechinger 39424* (G)	Iran	DQ357546
53. *Hesperidanthus argillaceus* (S. L. Welsh & N. D. Atwood) Al-Shehbaz [= *Schoenocrambe argillacea* (S. L. Welsh & N. D. Atwood) Rollins]	Warwick et al., 2002	U.S.A. (Utah)	AF531611‡
54. *Hesperis laciniata* All. [= *Hesperis laciniata* subsp. *spectabilis* (Jord.) Rouy & Foucaud]	Mummenhoff & Muehlhausen, unpubl. Isolate 9700821000	South Africa	AJ628315,‡ AJ628316‡
55. *Hesperis matronalis* L.*	*Merello et al. 2269* (MO)	Georgia	DQ357547
56. *Hesperis matronalis*	Franzke et al., 2004	—	AY546108,‡ AY546137‡
57. *Hesperis matronalis*	Mummenhoff & Muehlhausen, unpubl. Isolate 9400193000	South Africa	AJ628313,‡ AJ628314‡
58. *Hesperis sibirica* L.	*Stydzenkena s.n.*, 30 May 1997 (MO)	Russia (Siberia)	DQ357548
59. *Hesperis sibirica*	*Skvortsov & Blokhina s.n.*, 31 May 1983 (MO)	Russia	DQ357549
60. *Hesperis voronovii* N. Busch [= *Hesperis matronalis* subsp. *voronovii* (N. Busch) P. W. Ball]	*Merello et al. 2190* (MO)	Georgia	DQ357550
61. *Iskandera alaica* (Korsh.) Botsch. & Vved.	*Idarova & Garboshnova s.n.*, 3 June 1964 (LE)	Tajikistan	DQ357551
62. *Iskandera hissarica* N. Busch*	*Chukavina & Ameermukhamedov 10344* (LE)	Tajikistan	DQ357552
63. *Iskandera hissarica*	*Egorova 2252* (LE)	Tajikistan	DQ357553
64. *Leiospora eriocalyx* (Regel & Schmalh.) F. Dvorák	*Bartholomew et al. 8449* (MO)	China	DQ357554
65. *Leiospora exscapa* (C. A. Mey.) F. Dvorák*	Bleeker, Hurka, Oyuntsetseg et al., unpubl. ALTB B175	Russia (Altai Mtns.)	AY558939,‡ AY558967‡
66. *Leiospora pamirica* (Botsch. & Vved.) Botsch. & Pachom.	*Al-Shehbaz 8368* (MO)	China	DQ357555
67. *Leiospora pamirica*	Mummenhoff & Muehlhausen, unpubl. Isolate 1037	South Africa	AJ628329,‡ AJ628330‡

Table 3. Continued.

Taxon*	*Collector and No.* (Herbarium[a]); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
68. *Leptaleum filifolium* (Willd.) DC.*	*Rechinger 54714* (G)	Iran	DQ357556
69. *Malcolmia africana* (L.) R. Br. in W. T. Aiton	*Franklin 1354* (MO)	U.S.A. (Utah)	DQ357557
70. *Malcolmia africana*	*Harris 1892* (UVSC)	U.S.A. (Utah)	AY237307‡
71. *Malcolmia karelinii* Lipsky	*Al-Shehbaz 9405* (MO)	Kazakhstan	DQ357558
72. *Malcolmia littorea* (L.) R. Br. in W. T. Aiton	*Leadlay & Petty 376* (MO)	Portugal	DQ357559
73. *Malcolmia orsiniana* (Ten.) Ten.	*Evrard 10.828* (MO)	Greece	DQ357560
74. *Malcolmia triloba* (L.) Spreng. [= *Malcolmia lacera* (L.) DC.]	*Lewalle 13005* (MO)	Morocco	DQ357561
75. *Maresia nana* (DC.) Batt.*	*Raus & Schiers 16713* (MO)	Greece	DQ357562
76. *Mathewsia foliosa* Hook. & Arn.*	*Landrum & Landrum 9837* (MO)	Chile	DQ357563
77. *Matthiola capiomontana* (Durieu) Pomel [= *Lonchophora capiomontana* Durieu*]	*Faurel s.n.*, 10 May 1954 (G)	Algeria	DQ357564
78. *Matthiola farinosa* Bunge ex Boiss.	*Makhmedev & Zadorozhnuk 212* (MO)	Turkmenistan	DQ357565
79. *Matthiola fruticulosa* (L.) Maire	*Lewalle 9803* (MO)	Morocco	DQ357566
80. *Matthiola incana* (L.) R. Br.*	Mummenhoff & Muehlhausen, unpubl. Isolate 403	—	AJ628339,‡ AJ628340‡
81. *Matthiola lunata* DC.	Mummenhoff & Muehlhausen, unpubl. Isolate 355	—	AJ628341,‡ AJ628342‡
82. *Matthiola parviflora* (Schousb.) R. Br.	*Bramwell et al. 210* (MO)	Morocco	DQ357567
83. *Matthiola parviflora*	*Miller et al. 923* (MO)	Morocco	DQ357568
84. *Microstigma brachycarpum* Botsch.	*Liu & Zhang 93005* (MO)	China	DQ357569
85. *Morettia canescens* Boiss.	*Humbles 10073* (MO)	Saudi Arabia	DQ357570
86. *Morettia parviflora* Boiss.	*Gasperetti PG435* (MO)	Saudi Arabia	DQ357571
87. *Morettia philaeana* DC.*	*Goodman s.n.*, 28 Feb. 1985 (MO)	Egypt	DQ357572
88. *Neotorularia dentata* (Freyn & Sint.) Hedge & J. Léonard	Warwick et al., 2004a	Pakistan	AY353158‡
89. *Neotorularia korolkowii* (Regel & Schmalh.) Hedge & J. Léonard	Warwick et al., 2004a	Kazakhstan	AY353156‡
90. *Neotorularia torulosa* (Desf.) Hedge & J. Léonard*	Warwick et al., 2004a	Iran	AY353167‡
91. *Notoceras bicorne* (Aiton) Amo*	*Miller et al. 386* (MO)	Morocco	DQ357573
92. *Notothlaspi australe* Hook. f.*	Mitchell & Heenan, 2000	New Zealand	AF100689‡
93. *Notothlaspi rosulatum* Hook. f.	Mitchell & Heenan, 2000	New Zealand	AF100690‡
94. *Notothlaspi* sp. indet.	Mitchell & Heenan, 2000	New Zealand	AF110691‡
95. *Oreoloma matthioloides* (Franch.) Botsch.*	*Ma & Liu 83* (PE)	China	DQ357574
96. *Oreoloma sulfureum* Botsch.	*Potanin s.n.*, 13 May 1877 (LE)	China (Mongolia)	DQ357575
97. *Oreoloma violaceum* Botsch.	*Guan Kejia 574* (PE)	China	DQ357576
98. *Parolinia intermedia* Svent. & Bramwell	*Bramwell & Sventinius 1453* (MO)	Canary Islands	DQ357577
99. *Parrya asperrima* (B. Fedtsch.) Popov	*Botschantsev 438* (LE)	Kazakhstan	DQ357578
100. *Parrya pulvinata* M. Pop. in Komarov	*Botschantsev 202* (LE)	Kazakhstan	DQ357579
101. *Parrya turkestanica* (Korsh.) N. Busch	*Yunussov 6479* (LE)	Tajikistan	DQ357580
102. *Pseudocamelina glaucophylla* (DC.) N. Busch*	*Schmid 5420* (G)	Iran	DQ357581
103. *Pseudocamelina glaucophylla*	*Rechinger 47361* (G)	Iran	DQ357582
104. *Rhammatophyllum afghanicum* (Rech. f.) Al-Shehbaz & O. Appel	*Rechinger 36303* (B)	Afghanistan	DQ357583
105. *Rhammatophyllum frutex* Botsch. & Vved.	*Karamycheva et al. 4090* (LE)	Kazakhstan	DQ357584

Table 3. Continued.

Taxon*	*Collector and No.* (Herbarium[a]); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
106. *Rhammatophyllum gaudanense* (Litv.) Al-Shehbaz & O. Appel	*Nikitin et al. s.n.*, 18 June 1964 (LE)	Turkmenistan	DQ357585
107. *Rhammatophyllum ghoranum* (Rech. f.) Al-Shehbaz & O. Appel	*Palmer 110* (K)	Afghanistan	DQ357586
108. *Rhammatophyllum kamelinii* (Botsch.) Al-Shehbaz & O. Appel	*Kamelin & Darijmaa 331* (MO)	Mongolia	DQ357587
109. *Rhammatophyllum pachyrhizum* (Kar. & Kir.) O. E. Schulz*	*Kamaeshiva et al. 4178* (LE)	Kazakhstan	DQ357588
110. *Sisymbriopsis mollipila* (Maxim.) Botsch.	Warwick et al., 2004a	China	AY353157‡
111. *Sisymbriopsis yechengnica* (C. H. An) Al-Shehbaz et al.	Warwick et al., 2004a	China	AY353161‡
112. *Solms-laubachia eurycarpa* (Maxim.) Botsch.	*Ho et al. 2847* (MO)	China	DQ357589
113. *Solms-laubachia retropilosa* Botsch.	*Al-Shehbaz 9363* (MO)	China	DQ357590
114. *Sterigmostemum acanthocarpum* (Fisch. & C. A. Mey.) Kuntze	*Rechinger et al. 5318* (G)	Iran	DQ357591
115. *Sterigmostemum longistylum* (Boiss.) Kuntze	*Rechinger 46634* (G)	Iran	DQ357592
116. *Sterigmostemum purpurascens* (Boiss.) Kuntze	*Léonard 5793* (G)	Iran	DQ357593
117. *Sterigmostemum purpurascens*	*Rechinger 28025* (G)	Pakistan	DQ357594
118. *Sterigmostemum ramosissimum* (O. E. Schulz) Rech. f.	*Franskevitch s.n.*, 14 June 1972 (G)	Turkmenia	DQ357595
119. *Sterigmostemum ramosissimum*	*Rechinger 52318* (G)	Iran	DQ357596
120. *Sterigmostemum sulphureum* (Banks & Sol.) Bornm.	*Dep. Bot., U. Bagd. s.n.*, 20 Mar. 1958 (G)	Iraq	DQ357597
121. *Sterigmostemum sulphureum*	*Mouterde 8320* (G)	Lebanon	DQ357598
122. *Strigosella brevipes* (Bunge) Botsch.	Bleeker, Hurka, Oyuntsetseg, et al., unpubl. ALTB	Mongolia	AY558940,‡ AY558968‡
123. *Synstemon petrovii* Botsch.*	*Petrov s.n.*, 29 June 1958 (LE)	China	DQ357599
124. *Tetracme contorta* Boiss.	*Anders 5560* (G)	Afghanistan	DQ357600
125. *Tetracme contorta*	*Podlech 20408* (G)	Afghanistan	DQ357601
126. *Tetracme quadricornis* (Stephan) Bunge*	*Koelz 6194* (G)	India (Ladakh)	DQ357602
127. *Tetracme quadricornis*	*Rechinger 16836* (G)	Afghanistan	DQ357603
128. *Tetracme secunda* Boiss.	*Rechinger 33439* (G)	Afghanistan	DQ357604
129. *Zerdana anchonioides* Boiss.*	*Jacquemoud 7105* (G)	Iran	DQ357605
130. *Zuvanda crenulata* (DC.) R. K. Askerova	*Bertschinger s.n.*, 23 May 1935 (G)	Israel	DQ357606
131. *Zuvanda exacoides* (DC.) R. K. Askerova	*Pabot s.n.*, 11 May 1955 (G)	Lebanon	DQ357607
Arabideae			
132. *Arabis alpina* L.	O'Kane & Al-Shehbaz, 2003	Romania	AF137559‡
133. *Arabis blepharophylla* Hook. & Arn.	Koch et al., 1999a	—	AJ232903‡
134. *Arabis blepharophylla*	Koch et al., 1999a	—	AJ232904‡
135. *Arabis hirsuta* (L.) Scop.	Koch et al., 1999a	—	AJ232885‡
136. *Arabis hirsuta*	Koch et al., 1999a	—	AJ232886‡
137. *Arabis procurrens* Waldst. & Kit.	Koch et al., 1999a	—	AJ232916‡
138. *Arabis procurrens*	Koch et al., 1999a	—	AJ232917‡
139. *Pseudoturritis turrita* (L.) Al-Shehbaz	Koch et al., 1999a	—	AJ232905‡
140. *Pseudoturritis turrita*	Koch et al., 1999a	—	AJ232906‡
Cardamineae			
141. *Cardamine flexuosa* With.	Franzke et al., 1998	—	AF077999,‡ AF0778000‡
142. *Rorippa palustris* (L.) Besser	Franzke et al., 1998	—	AF078021,‡ AF078022‡

Table 3. Continued.

Taxon*	*Collector and No.* (Herbarium[a]); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
Camelineae			
143. *Arabidopsis thaliana* (L.) Heynh.	O'Kane et al., 1997	Afghanistan	ATU43225‡
144. *Neslia paniculata* (L.) Desv.*	O'Kane & Al-Shehbaz, 2003	—	AF137576‡
Cochlearieae			
145. *Cochlearia megalosperma* (Maire) R. Vogt	Koch et al., 1999b	—	AF336208.‡ AF336209‡
146. *Ionopsidium acaule* Rchb.	Koch et al., 1999b	—	AF336210.‡ AF336211‡
Schizopetaleae			
147. *Sisymbrium castellanosii* O. E. Schulz	Warwick et al., 2006a	Argentina	AY958592‡
148. *Thelypodiopsis elegans* Rydb.*	Warwick et al., 2002	U.S.A. (Colorado)	AF531638‡
149. *Thelypodium flexuosum* B. L. Rob.	Warwick et al., 2002	U.S.A. (Nevada)	AF531624‡
150. *Weberbauera peruviana* (DC.) Al-Shehbaz	Warwick et al., 2002	Bolivia	AF531593‡
Sisymbrieae			
151. *Sisymbrium irio* L.	Warwick et al., 2002	Belgium	AF534558‡
Thlaspideae			
152. *Thlaspi arvense* L.	Koch & Mummenhoff, 2001	—	AF336152.‡ AF336153‡
OUTGROUP			
Aethionemeae			
153. *Aethionema arabicum* (L.) Andrz. ex O. E. Schulz	Hong et al., 2003	—	AY254539‡

* Type species.
[a] Herbarium acronyms (Holmgren et al., 1990).
‡ Previously published sequences, citation given in second column.

amount of homoplasy in the trees, described in PAUP*, were obtained for each tree, and a strict consensus tree was computed. A total of 1000 bootstrap (Felsenstein, 1985) replicates, respectively, were generated in PAUP* using a full heuristic search, with options ACCTRAN, MULTREES=MULPARS, and TBR branch-swapping, and each replicate generated with simple addition sequence of taxa, to test the stability of particular nodes in the parsimony analysis. Pairwise distance ITS sequence divergence was calculated for each accession pair in PAUP* using the Kimura two-parameter model (Kimura, 1980) and the pairwise deletion option for gaps and ambiguous data.

Fifty-six sequence evolution models for maximum-likelihood analyses were tested with the aid of Modeltest v3.08 (Posada & Crandall, 1998) to establish which model of DNA substitution best fits the data. This program compares these maximum-likelihood models in a hierarchical testing framework by calculating the likelihood ratio statistic between different models. Likelihood ratio tests and their associated *P* value compare alternative models of sequence evolution and improvement in fit with increasing model complexity. A heuristic maximum-likelihood search was then conducted in PAUP* using parameters determined for the best model of sequence evolution, "as is" sequence addition, and TBR swapping.

Results

A total of 119 species (153 accessions), including the outgroup, were used in the analysis (Table 3). The resulting multiple alignment of the ITS region, including the 5.8S gene, was 869 bp long. Of these, 398 bp were parsimony uninformative, 108 bp were variable but not parsimony informative, and 363 bp were potentially parsimony informative. The ITS-1 region was variable at 261 of 444 sites (58.8%), the ITS-2 at 190 of 260 sites (73.1%), and the 5.8S gene at only 20 of the 165 sites (12.1%). The alignment required the introduction of several indels, most of which were 1 to 3 bp, with the exception of a 108 bp insertion for *Sterigmostemum acanthocarpum* (Fisch. & C. A. Mey.) Kuntze and *S. sulphureum* (Banks & Soland.) Bornm.; a 27 bp deletion in ITS-1 for *Aubrieta* Adans. species; a 7 bp insert in ITS-2 for *Malcolmia triloba* (L.) Spreng. and *M. littorea* (L.) R. Br.; a 6 bp deletion in ITS-1 for *Anchonium* DC., *Oreoloma*, *Zerdana* Boiss., *Synstemon* Botsch., and

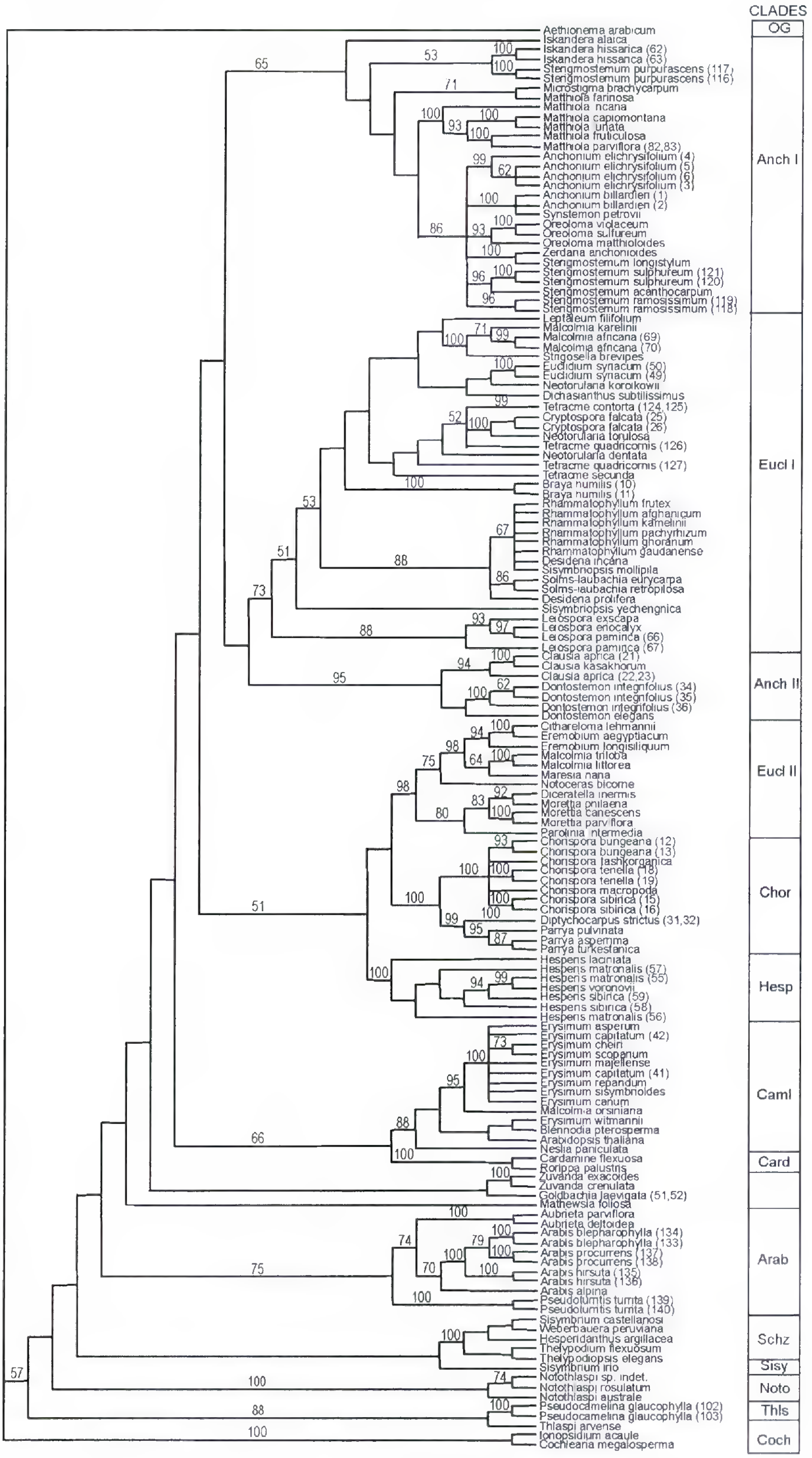

Figure 1. Strict consensus tree of the 34,500 most parsimonious trees generated from analysis in PAUP* of 148 ITS sequences from 121 taxa. Tree length = 2846 (based on 363 parsimony informative characters); consistency index (CI) = 0.273; retention index (RI) = 0.741. Bootstrap values with over 50% support appear above branches. The numbers in parentheses reflect taxon/species ordering in Table 3; all conspecific samples listed within parentheses had identical sequences. Major clades are indicated to the right and include from top to bottom: Outgroup (OG), Anchonieae I (Anch I), Euclidieae I (Eucl I), Anchonieae II (Anch II), Euclidieae II (Eucl II), Chorisporeae (Chor), Hesperideae (Hesp), Camelineae (Caml), Cardamineae (Card), Arabideae (Arab), Schizopetaleae (Schz), Sisymbrieae (Sisy), *Notothlaspi* (Noto), Thlaspideae (Thls), and Cochlearieae (Coch).

Sterigmostemum species (excluding *S. purpurascens* (Boiss.) Kuntze); a 6 bp insert in ITS-1 for *Chorispora* species; a 33 bp deletion in ITS-1 for *Diptychocarpus* accessions; a 11 bp deletion for *Parrya* species; a 14 bp and 12 bp insertion in ITS-2 for *Chorispora*, *Diptychocarpus*, and *Parrya*; and a 7 bp insertion in ITS-2 for *Chorispora* and *Parrya*. The length of the ITS region in the ingroup taxa ranged from 597 bp for *Aubrieta* species to 727 bp for *Sterigmostemum acanthocarpum*. The G + C content of taxa in the ingroup averaged 55%.

The equal weight maximum parsimony analysis, based on 148 ITS sequences (each representing a unique sequence) and 363 informative characters, yielded 550 most parsimonious trees of 2846 steps. When the 50% majority-rule tree of the above 550 trees was used as the starting tree in a subsequent heuristic search and then swapped to completion, a total of 34,500 most parsimonious trees were obtained. The strict consensus trees of both analyses were the same. Figure 1 shows the strict consensus tree, with bootstrap values. Several well-supported clades were observed and are listed below in the order they appear in the figure: Anchonieae I (Anch I), Euclidieae I (Eucl I), Anchonieae II (Anch II), Euclidieae II (Eucl II), Chorisporeae (Chor), Hesperideae (Hesp), Camelineae (Caml), Cardamineae (Card), Arabideae (Arab), Schizopetaleae (Schz), Sisymbrieae (Sisy), *Notothlaspi* Hook. f. (Noto), Thlaspideae (Thls), and Cochlearieae (Coch) (Fig. 1). Figure 2 shows one of the most parsimonious trees, with the number of characters supporting each clade indicated.

Hierarchical likelihood ratio tests indicated that the best model of DNA substitution for the ITS data is TrN + I + G, which allows for unequal base frequencies, two substitution rates for transitions, one substitution rate for transversions, among site rate heterogeneity allowing some sites to be invariant (I = 0.272), while the rest have rates drawn from a discrete approximation to a gamma distribution, with a single shape parameter, alpha (G = 0.9274). With the TrN + I + G model, base frequencies for A = 0.2597, C = 0.2399, G = 0.2078, T = 0.2926, and substitution rates A-C, A-T, C-G, and G-T = 1.0000, A-G = 2.3031, and C-T = 3.2256. The heuristic search produced a single tree (-ln L = 15673.5) shown in Figure 3 that is nearly identical in topology to the consensus tree of the parsimony analysis. The same major clades were evident, with only minor differences in the positions of *Goldbachia* DC., *Zuvanda* (Dvorák) Askerova, and *Mathewsia* Hook. & Arn. relative to each other. Their tribal positions were also not resolved in the PAUP* analysis.

ITS sequence divergence estimates among species (e.g., corrected pairwise distance values based on the Kimura two-parameter model) are given in Table 4. Within-clade estimates ranged from 0.33% to 23.5% (mean 11.3%) for the Anchonieae I clade, from 1.6% to 13.3% (mean 8.3%) for the Anchonieae II clade, from 0.66% to 18.7% (mean 7.3%) for the Euclidieae I clade, from 0.16% to 18.4% (mean 11.9%) for the Euclidieae II clade, from 1.99% to 12.9% (mean 7.8%) for the Chorisporeae clade, and from 2.8% to 8.7% (mean 5.8%) for the Hesperideae clade. The mean sequence divergence estimates among these six clades were similar, ranging from 13.1% to 20.4%.

In general, multiple accessions of a given species formed single clades. Eleven of 24 genera, where more than two congeneric species were analyzed, were monophyletic (Fig. 1); these included *Aubrieta*, *Chorispora*, *Clausia* Korn.-Trotzky, *Dontostemon*, *Hesperis*, *Leiospora* (C. A. Mey.) F. Dvorák, *Notothlaspi*, *Oreoloma*, *Parrya*, *Solms-laubachia*, and *Zuvanda*.

Discussion

Tribes/Clades

The major clades recognized in the ITS analyses correspond very closely to *ndh*F-based clades described in Beilstein et al. (2006), which were used as a basis for a new tribal system for the family. Our results are discussed relative to the corresponding tribal system outlined in Al-Shehbaz et al. (2006).

Anchonieae I

This ITS clade (Figs. 1–3) included *Anchonium*, *Iskandera* N. Busch, *Matthiola*, *Microstigma* Trautv., *Oreoloma*, *Sterigmostemum*, *Synstemon*, and *Zerdana*. The clade was divided into three main subclades: (i) *Anchonium*, *Oreoloma*, *Sterigmostemum* (excluding *S. purpurascens*), *Synstemon*, and *Zerdana* (86% bootstrap support); (ii) *Matthiola*, excluding *M. farinosa* Bunge ex Boiss. (100% bootstrap support); and (iii) *Microstigma* and *Matthiola farinosa* (71% bootstrap support) (Fig. 1). *Matthiola* formed a monophyletic group, with the exclusion of *M. farinosa*. The placement of *Lonchophora* Durieu (represented in this study by *M. capiomontana* (Durieu) Pomel) in synonymy of *Matthiola* (Gowler, 1998; Appel & Al-Shehbaz, 2003) was supported by the ITS data. *Sterigmostemum*, as defined by Jacquemoud (1988), is polyphyletic, with *S. purpurascens* forming a sister taxon to *Iskandera*. The latter species, an annual endemic to Iran and Pakistan, was considered to be segregated from the rest of *Sterigmostemum* (Jacquemoud, 1988) based on the presence of peculiar subglobose glands, white or pale pink flowers, and (as in *Iskandera*) free inner stamens. Nevertheless,

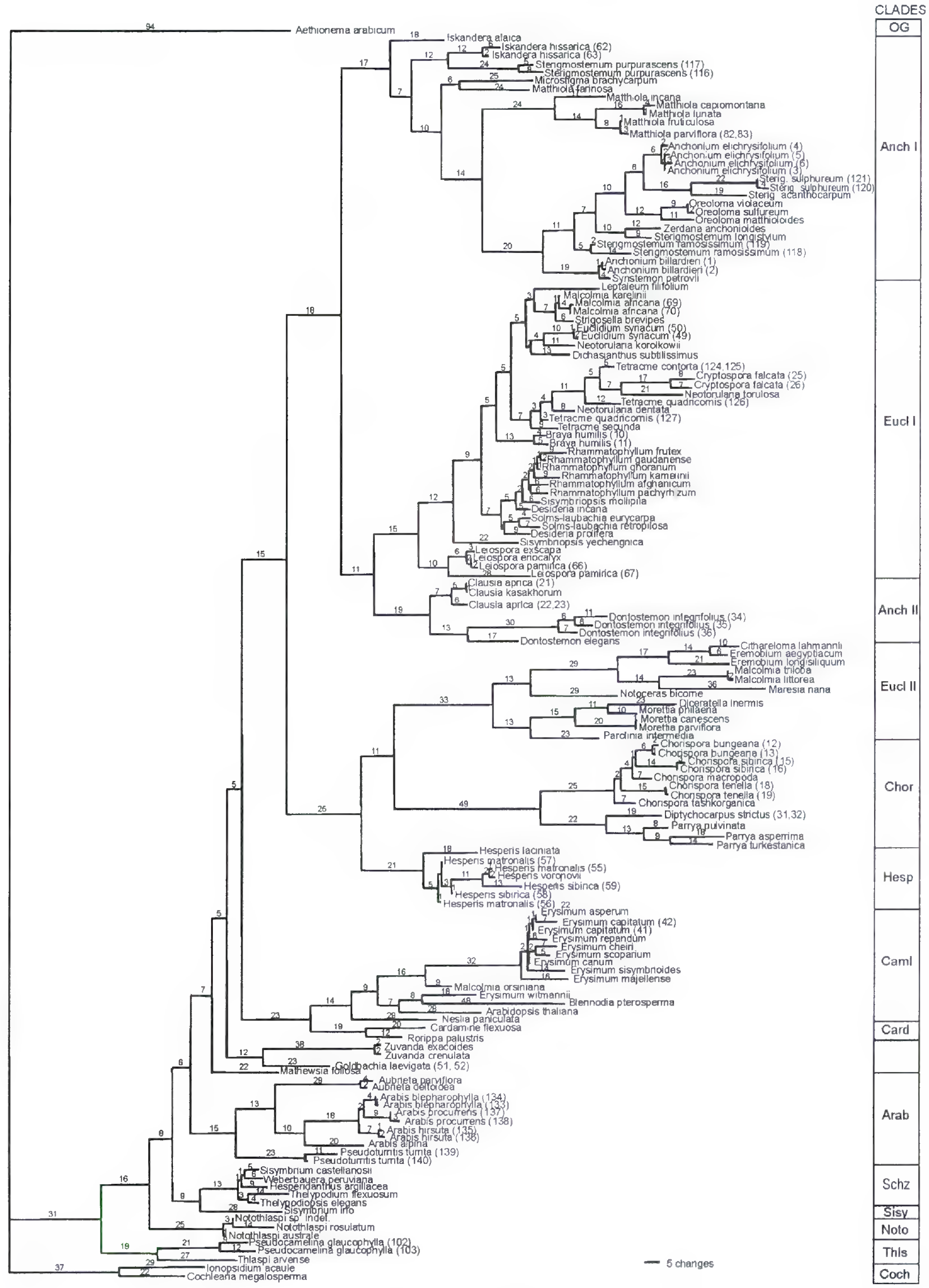

Figure 2. One of the most parsimonious trees, tree length = 2846 (based on 363 parsimony informative characters); CI = 0.273; RI = 0.741. The number of characters supporting each clade is indicated. The numbers in parentheses reflect taxon/species ordering in Table 3; all conspecific samples listed within parentheses had identical sequences. Major clades are indicated to the right and include from top to bottom: Outgroup (OG), Anchonieae I (Anch I), Euclidieae I (Eucl I), Anchonieae II (Anch II), Euclidieae II (Eucl II), Chorisporeae (Chor), Hesperideae (Hesp), Camelineae (Caml), Cardamineae (Card), Arabideae (Arab), Schizopetaleae (Schz), Sisymbrieae (Sisy), *Notothlaspi* (Noto), Thlaspideae (Thls), and Cochlearieae (Coch). *Sterig.* = *Sterigmostemum*.

Figure 3. Maximum likelihood tree generated under the TrN + I + G model of DNA substitution in PAUP* in an analysis of 148 ITS sequences from 121 taxa. The numbers in parentheses reflect taxon/species ordering in Table 3; all conspecific samples listed within parentheses had identical sequences. Major clades are indicated to the right and include: Outgroup (OG), Anchonieae I (Anch I), Euclidieae I (Eucl I), Anchonieae II (Anch II), Camelineae (Caml), Cardamineae (Card), Arabideae (Arab), Euclidieae II (Eucl II), Chorisporeae (Chor), Hesperideae (Hesp), *Notothlaspi* (Noto), Cochlearieae (Coch), Schizopetaleae (Schz), Sisymbrieae (Sisy), and Thlaspideae (Thls). *Sterig.* = *Sterigmostemum*. The topology of the tree is almost identical to the strict consensus tree (Fig. 1) of the most parsimonious trees with the exception of the relative positions of *Goldbachia*, *Zuvanda*, and *Mathewsia*, whose tribal alignment was also not resolved in the PAUP* analysis.

Table 4. Mean (range) sequence divergence estimates (i.e., corrected pairwise distance values based on Kimura two-parameter model) between and within six clades observed in the present study: Anchonieae I and II, Euclidieae I and II, Chorisporeae, and the Hesperideae.

	Anchonieae I	Anchonieae II	Euclidieae I	Euclidieae II	Chorisporeae	Hesperideae
Anchonieae I	0.1129 (0.0033–0.2345)					
Anchonieae II	0.1568 (0.1061–0.2581)	0.0829 (0.0161–0.1326)				
Euclidieae I	0.1308 (0.0170–0.2724)	0.1398 (0.0990–0.2186)	0.0725 (0.0066–0.1873)			
Euclidieae II	0.1966 (0.1529–0.3357)	0.2026 (0.1712–0.3153)	0.1960 (0.1464–0.3047)	0.1190 (0.0016–0.1843)		
Chorisporeae	0.1906 (0.1543–0.3045)	0.1971 (0.1664–0.2567)	0.2016 (0.1536–0.3093)	0.1986 (0.1710–0.2488)	0.0779 (0.0199–0.1289)	
Hesperideae	0.1947 (0.1267–0.2702)	0.1879 (0.1505–0.2340)	0.1942 (0.1424–0.2493)	0.2040 (0.1423–0.3154)	0.1960 (0.1465–0.2762)	0.0580 (0.0282–0.0869)

trichome morphology, chorology, ecology, habit, and moreover, the lack of affinities with any genus but *Sterigmostemum* within the Hesperidae-Matthiolae group led this author to keep the species in *Sterigmostemum*. In contrast, Léonard (1980) used the peculiarity of *S. purpurascens* as the basis for its placement in the monotypic *Petiniotia* J. Léonard. The ITS data clearly support a closer affiliation of the species with *Iskandera* than with *Sterigmostemum*. The remainder of the *Sterigmostemum* taxa were included in a subclade, with *Anchonium*, *Oreoloma*, *Synstemon*, and *Zerdana*. *Oreoloma*, a genus of three species from China and Mongolia (Botschantzev, 1980) all of which were examined here, formed a monophyletic group (Fig. 1), although the genus has recently been united with *Sterigmostemum* (Kamelin & German, 2001). *Synstemon*, another Chinese endemic (2 species; Al-Shehbaz et al., 2000b), formed a sister group to *Anchonium billardieri* DC. *Zerdana*, a west Iranian endemic (1 species; Jacquemoud, 1985), formed a sister group to *Sterigmostemum longistylum* (Boiss.) Kuntze. The Middle Eastern *Anchonium* (2 species; Jacquemoud, 1984) did not form a monophyletic group.

Although the Anchonieae I has only 65% bootstrap support (Fig. 1), it corresponds well to the Anchonieae sensu Al-Shehbaz et al. (2006), except that it excludes *Dontostemon*, *Parrya*, and *Clausia*. The additional adjustments needed in this tribe are perhaps to transfer *Matthiola farinosa* to *Microstigma* and to recognize the genus *Petiniotia* as distinct from *Sterigmostemum*. Furthermore, contrary to their union in one genus (Kamelin & German, 2001), *Oreoloma* should continue to be maintained as distinct from *Sterigmostemum*, in particular on the basis of its floral morphology (petals longly clawed, lateral sepals saccate at the base). Indumentum morphology and inner stamens status also show close affinities within the Anchonieae: only members of *Anchonium*, *Oreoloma*, *Sterigmostemum* s. str., and *Zerdana* share the simultaneous occurrence of branched hairs, stalked glands, inner connate stamens, and nothorhizous embryo, whereas *Iskandera* only shares with them branched hairs, stalked glands, and nothorhizous embryo (Jacquemoud, 1988).

Chromosome reports indicated a wide range of numbers for members in this clade, with $x = 5, 6, 7, 8$, and 13 (Table 2; Warwick & Al-Shehbaz, 2006).

ANCHONIEAE II

Clausia (6 species; central Asia, southeastern Europe, and Middle East) and *Dontostemon* (11 species, eastern and central Asia; Al-Shehbaz & Ohba, 2000), which were assigned to the Anchonieae in Al-Shehbaz et al. (2006), formed a well-supported ITS clade (95% support in Fig. 1) distinctly separate from the other Anchonieae. Both genera were monophyletic (Fig. 1) and have a base chromosome number of $x = 7$ (Table 2; Warwick & Al-Shehbaz, 2006). The group would appear to represent a distinct tribe. The central Asian *Pseudoclausia* Popov should be studied in connection with these two genera because of their marked morphological similarities (Appel & Al-Shehbaz, 2003).

EUCLIDIEAE I

This clade (see Figs. 1–3) included *Braya* (13 species; Warwick et al., 2004a), *Cryptospora* Kar. & Kir. (3 species; Botschantzev, 1963b), *Desideria* (12 species; Al-Shehbaz, 2000), *Dichasianthus* Ovcz. & Junussov (1 species), *Euclidium* (2 species), *Leiospora* (6 species), *Leptaleum* DC. (1 species), *Neotorularia* (11 species; Warwick et al., 2004a), *Rhammatophyllum* (10 species; Al-Shehbaz & Appel, 2002), *Sisymbriopsis* (3 species; Al-Shehbaz et al., 1999), *Solms-laubachia* (9 species; Lan & Cheo, 1981; Al-Shehbaz & Yang, 2001; Yue et al., 2006), *Strigosella* Boiss. (as *Malcolmia africana* (L.) R. Br. and *M. karelinii* Lipsky), and *Tetracme* (10 species). All of these were assigned to the Euclidieae by Al-Shehbaz et al. (2006). Previous studies (Warwick et al., 2004a) supported the close relationships among *Braya*, *Dichasianthus*, *Neotorularia* s.l., and *Sisymbriopsis*, and these genera fell in the same tribe defined by Al-Shehbaz et al. (2006) as the Euclidieae. Base chromosome numbers for genera in this clade are $x = 7$ or 14, with the exception of *Rhammatophyllum* with $x = 8$ (Table 2; Warwick & Al-Shehbaz, 2006).

Desideria, *Rhammatophyllum*, *Solms-laubachia*, and *Sisymbriopsis mollipila* (Maxim.) Botsch. formed a well-supported subclade (88% bootstrap support) within the Euclidieae I clade (Figs. 1–3). A similarly well-supported clade (95% bootstrap support) was observed for these four genera in the *ndh*F-based studies of Beilstein et al. (2006). In recent *mat*K and *Chs*-based studies (Yue et al., 2006), *Desideria* and *Solms-laubachia* also formed a well-supported clade (100% bootstrap support), along with *Phaeonychium jafrii* Al-Shehbaz; neither *Desideria* nor *Solms-laubachia* were monophyletic, leading these authors to suggest that *Solms-laubachia* be expanded to include *Desideria* and *P. jafrii*. These and the ITS data presented herein suggest that further studies on the generic limits of these related genera are required.

Although the genera *Parolinia* Webb, *Maresia* Pomel, *Morettia* DC., *Chithareloma* Bunge, *Eremobium* Boiss., and *Notoceras* R. Br. were suggested by Al-Shehbaz et al. (2006) to be possible representatives of the Euclidieae, our results clearly support their removal with the Mediterranean *Malcolmia* (including the generic type) into a separate tribal clade (see below). Our studies also support the splitting of both *Sisymbriopsis* sensu Al-Shehbaz et al. (1999) and *Neotorularia* (Léonard, 1986) into at least two genera (for further details, see Warwick et al., 2004a). *Strigosella brevipes* (Bunge) Botsch. formed a well-supported clade with *Malcolmia africana* and *M. karelinii* that was separate from the clade containing European/Mediterranean taxa of *Malcolmia*, which are the most likely sister species to the type of *Malcolmia* (*M. maritima* (L.) R. Br., material not included). The latter taxa fell outside the Euclidieae as defined herein, whereas those placed in *Strigosella* sensu Botschantzev (1972b) were retained in that tribe. Therefore, our data strongly support the independent recognition of *Strigosella*, a genus reduced to synonymy of *Malcolmia* by nearly all except a few authors (e.g., Townsend, 1980; Czerepanov, 1995). Our results clearly show that the two genera are not closely related and should be assigned to different tribes. *Strigosella* is primarily central and southwest Asian and has non-saccate lateral sepals, stalked and dendritic or forked trichomes, and a base chromosome number of $x = 7$ (Warwick & Al-Shehbaz, 2006). By contrast, *Malcolmia* is Mediterranean and has strongly saccate lateral sepals, sessile stellate or malpighiaceous trichomes, and base chromosome numbers of $x = 8$ or 10 (Warwick & Al-Shehbaz, 2006).

EUCLIDIEAE II

As shown in Figures 1 and 2 and discussed above, the Euclidieae II clade was well defined with 98% bootstrap support, and unrelated to other Euclidieae. It separated into two subclades: the first with 75% bootstrap support included *Chithareloma* (Jafri, 1973), *Eremobium*, *Malcolmia*, *Maresia*, and *Notoceras*, whereas the second subclade had 80% bootstrap support and included the African genera *Diceratella* Boiss. (11 species; Jonsell, 1979), *Morettia* (3 species; Jonsell, 1979; Stork & Wüest, 1980), and *Parolinia* (5 species; Bramwell, 1970). All genera of Euclidieae II were tentatively assigned to the Euclidieae in Al-Shehbaz et al. (2006), but they clearly form a distinct clade separate from the other Euclidieae. Prantl (1891) placed all of the above genera in the tribe Hesperideae subtribe Malcolmiinae, but it is clear that the group should be kept as a distinct tribe, with subtribe Malcolmiinae raised to the tribal rank. Further studies are needed to establish if the two subclades of Euclidieae II merit a subtribal rank.

All three genera in the African subclade have a base chromosome number of $x = 11$; whereas chromsome numbers for the second Eurasian subclade are more variable with $x = 7, 8, 10, 11, 13, 14$ (Table 2; Warwick & Al-Shehbaz, 2006).

CHORISPOREAE

This clade included *Chorispora*, *Diptychocarpus*, and *Parrya* (Figs. 1–3). Both *Chorispora* and *Parrya* formed monophyletic groups. The ITS data are consistent with the separate recognition of this tribe by Al-Shehbaz et al. (2006), which included *Chorispora* (11 species; Al-Shehbaz et al., 2000a) and

Diptychocarpus (1 species). In the *ndh*F-based phylogenetic study of Beilstein et al. (2006), *Chorispora* (1 species) and *Diptychocarpus* (1 species) formed a well-supported clade. *Parrya*, a primarily central Asian genus of ca. 34 species (Botschantzev, 1972a), was not included in the *ndh*F study. Its placement in the Anchonieae with other genera having multicellular-multiseriate glands was recommended by Al-Shehbaz et al. (2006), but ITS analyses clearly support its reassignment to the Chorisporeae. As concluded herein, the genus is firmly assigned to the tribe Chorisporeae instead of the Anchonieae or other tribes. All three genera in this tribe have a base chromosome number of $x = 7$ (Table 2; Warwick & Al-Shehbaz, 2006).

HESPERIDEAE

Hesperis formed a well-supported clade (Figs. 1–3), consistent with its assignment to a separate unigeneric tribe, the Hesperideae, by Al-Shehbaz et al. (2006). As delimited by Prantl (1891), Hayek (1911), and Schulz (1936), the Hesperideae had included many other genera, none of which have uniseriate glands, and de Candolle (1821) had even placed *Hesperis* in the Sisymbrieae. The genus *Hesperis* (46 species) is centered in the Middle East and Europe, with poor representations in northern Africa and central Asia. Chromosome number reports are highly variable for the genus (Table 2; Warwick & Al-Shehbaz, 2006).

TAXA EXCLUDED FROM THE ANCHONIEAE, CHORISPOREAE, EUCLIDIEAE, AND HESPERIDEAE

The studies herein support the exclusion of *Aubrieta*, *Blennodia* R. Br., *Erysimum*, *Goldbachia*, *Hesperidanthus* (B. L. Rob.) Rydb., *Notothlaspi*, *Pseudocamelina* (Boiss.) N. Busch, and *Zuvanda* from the Anchonieae, Chorisporeae, Euclidieae, and Hesperideae. *Aubrieta*, originally assigned to the Matthioleae by Schulz (1936), formed herein a sister clade to *Arabis* L. (Figs. 1–3), consistent with its reassignment to the tribe Arabideae by Janchen (1942) and Al-Shehbaz et al. (2006). Both *Aubrieta* and *Arabis* have base chromosome numbers of $x = 8$ (Warwick & Al-Shehbaz, 2006). Placement of *Aubrieta* in the Arabideae is also consistent with previous ITS-based studies of *A. deltoidea* (L.) DC. (Bailey et al., 2006), *ndh*F-based phylogenetic studies of *A. deltoidea* and *A. parviflora* Boiss. (Beilstein et al., 2006), and the alignment of *A. deltoidea* in a supernetwork multigene-based analysis of the family (Koch et al., 2007).

The Australian genus *Blennodia* was originally placed in the Hesperideae by Schulz (1936) and maintained there by Shaw (1965). Al-Shehbaz (1988) indicated that it should be excluded from this tribe, but its placement is problematic and, thus, was not assigned in Al-Shehbaz et al. (2006). It aligned herein with tribe Camelineae (Fig. 1), forming a loosely supported (less than 50%) clade with *Arabidopsis thaliana* and *Erysimum witmannii* Zaw. in the present analyses.

Erysimum was assigned to the Hesperideae by Schulz (1936) and Janchen (1942), to the Anchonieae by Al-Shehbaz (1988), and later to the Camelineae, along with *Arabidopsis* (DC.) Heynh., by Al-Shehbaz et al. (2006). Placement of *Erysimum* in the Camelineae as observed in the present study (Figs. 1–3) is also consistent with previous ITS (Bailey et al., 2006) and *ndh*F data (Beilstein et al., 2006) for *E. capitatum* (Douglas ex Hook.) Greene. *Erysimum* (with the exception of *E. witmannii*) formed a monophyletic group (100% bootstrap support; Fig. 1), with the inclusion of *Malcolmia orsiniana* (Ten.) Ten. The latter specimen would appear to be correctly identified (as confirmed by Al-Shehbaz), and its inclusion with *Erysimum* requires further analysis of additional accessions. The inclusion in *Erysimum* of *Cheiranthus* L. and *Syrenia* Andrz. ex Besser, represented in this study by *E. cheiri* (L.) Crantz and *E. canum* (Piller & Mitterp.) Polatschek, respectively, was supported by the ITS data herein. *Neslia* Desv. (1 species), assigned to the Camelineae in Al-Shehbaz et al. (2006), was closely related to *Erysimum* and *Arabidopsis* and is retained herein in that tribe.

Goldbachia, a Eurasian genus of six species (Botschantzev, 1963a), and *Zuvanda*, a southwest Asian genus of three species (Askerova, 1985), were sister clades in the strict consensus tree shown in Figure 1; the latter clade, however, had less than 50% bootstrap support. Both were placed in the Hesperideae by Schulz (1936) and later in the subtribe Buniadinae DC. of the Sisymbrieae by Janchen (1942), but they were not assigned in Al-Shehbaz et al. (2006). Taxa currently assigned to *Zuvanda* were included in *Malcolmia* (Schulz, 1936), later placed in *Maresia* subgenus *Zuvanda* (Dvorák, 1972), then treated as a separate genus by Askerova (1985), and lastly united with *Moricandia* DC. of the totally unrelated tribe Brassiceae by Dorofeyev (2002). The exclusion of *Zuvanda* from both *Malcolmia* and *Maresia* was supported by our ITS data (Figs. 1–3). The tribal positions of *Goldbachia* and *Zuvanda*, however, are unresolved in the present study; the tribal position of *Goldbachia laevigata* (M. Bieb.) DC. also was not resolved in recent *ndh*F-based phylogenetic studies (Beilstein et al., 2006). Both *Zuvanda* and *Goldbachia* are glabrous and somewhat glaucous annuals with auriculate cauline leaves. They need to be closely studied with members of the tribe Isatideae DC. to determine whether they merit a tribal rank of their own.

Hesperidanthus (5 species), originally assigned to Matthioleae in Schulz (1936) and to Hesperideae subtribe Matthiolinae in Janchen (1942), formed a clade with the New World *Sisymbrium*, *Thelypodium* Endl., *Thelypodiopsis* Rydb., and *Weberbauera* Gilg & Muschl., all members of the tribe Schizopetaleae (Al-Shehbaz et al., 2006; = Thelypodieae Alliance in Warwick et al., 2002, 2006b). The ITS data, herein for *H. argillaceus* (S. L. Welsch & N. D. Atwood) Al-Shehbaz, thus support its assignment to that tribe by Al-Shehbaz et al. (2006). Placement of *Hesperidanthus* in the Schizopetaleae is also consistent with previous ITS data for *H. argillaceus* and *H. linearifolius* (A. Gray) Rydb. (Bailey et al., 2006) and *ndh*F data (Beilstein et al., 2006) for *H. jaegeri* (Rollins) Al-Shehbaz [= *Caulostramina jaegeri* (Rollins) Rollins] and *H. suffrutescens* (Rollins) Al-Shehbaz [= *Glaucocarpum suffrutescens* (Rollins) Rollins]. In contrast, *Mathewsia foliosa* Hook & Arn. did not align with the Schizopetaleae (Al-Shehbaz et al., 2006) or any other tribal clade in the present analyses (Figs. 1–3). The genus *Mathewsia* (10 species) is endemic to South America and uniquely occupies desert habitats. Its generic affinities have been difficult to define (Rollins, 1966) and remain so. A better sampling of the genus, along with several South American genera of the Brassicaceae, is needed to determine its tribal assignment.

Notothlaspi (2 species), a genus endemic to New Zealand, was tentatively assigned to the Euclidieae (Al-Shehbaz et al., 2006) but did not align with any of the tribal clades in this analysis (Figs. 1–3). It had been previously assigned to the Lepidieae (Schulz, 1936; Janchen, 1942). Previous ITS-based molecular studies (Mitchell & Heenan, 2000; Heenan et al., 2002; Bailey et al., 2006) also showed that *Notothlaspi* formed a distinct monophyletic group that was not clearly aligned with any of the tribes studied, including the Lepidieae. The genus has an unusually high chromosome number of $x = 19$ (Table 2; Warwick & Al-Shehbaz, 2006), which would suggest polyploid origins. This genus needs to be studied closely in connection with genera that have similar morphologies, such as *Grammosperma* O. E. Schulz and *Carinavalva* Ising; all three have angustiseptate, many-seeded fruits, and a Southern Hemisphere distribution.

Pseudocamelina, an Iranian genus of three species (Miller, 1978; Appel & Al-Shehbaz, 2003), was originally assigned to the Matthioleae by Schulz (1936) and to the Lepidieae by Janchen (1942). It formed a well-supported clade in Figure 1 with *Thlaspi* L., consistent with its assignment to the Thlaspideae in Al-Shehbaz et al. (2006) and its tribal placement based on *ndh*F-based studies of *Pseudocamelina campylopoda* Bornm. & Gauba ex Bornm. (Beilstein et al., 2006).

GENERIC MONOPHYLY

Eleven of the 24 genera where more than two congeneric species were analyzed were monophyletic; these included *Aubrieta*, *Chorispora*, *Clausia*, *Dontostemon*, *Hesperis*, *Leiospora*, *Notothlaspi*, *Oreoloma*, *Parrya*, *Solms-laubachia*, and *Zuvanda*. Twelve genera, *Anchonium*, *Desideria*, *Eremobium*, *Erysimum*, *Iskandera*, *Matthiola*, *Morettia*, *Neotorularia*, *Rhammatophyllum*, *Sisymbriopsis*, *Sterigmostemum*, and *Tetracme*, each fell within a single tribal clade but were not monophyletic. The genus *Malcolmia*, ca. 35 species, was, however, paraphyletic across tribal clades. The species were separated into three clades:

(i) "*Strigosella* subclade"

The two central Asian species, *Malcolmia africana* and *M. karelinii*, formed a distinct clade (100% bootstrap support) with *Strigosella brevipes* and other eastern and central Asian species in the Euclidieae I clade (Fig. 1), including *Neotorularia korolkowii* (Regel & Schmalh.) Hedge & J. Léonard and *Leptaleum filifolium* (Willd.) DC. *Strigosella* (Boissier, 1854) was included in the synonymy of *Malcolmia* in Appel and Al-Shehbaz (2003). The ITS data herein are consistent with its separate recognition from *Malcolmia*.

(ii) "*Erysimum* subclade"

Malcolmia orsiniana, a European species restricted to the mountains of the Balkan peninsula, was included in *Erysimum* in the tribe Camelineae clade (Fig. 1). Its inclusion with *Erysimum* requires further analysis of additional accessions.

(iii) "*Malcolmia* subclade"

The two southwestern European taxa, *Malcolmia triloba* (L.) Spreng. and *M. littorea* (L.) R. Br., were sister taxa to *Maresia nana* (DC.) Batt. (= *Malcolmia nana* (DC.) Boiss., and treated as such by Greuter et al., 1986). The three taxa formed a clade with *Citharelonia*, *Eremobium*, and *Notoceras* in a distinctly separate clade (75% bootstrap support) within the Euclidieae II clade (Fig. 1).

SEQUENCE DIVERGENCE

The mean sequence divergence estimates among the six clades, Anchonieae I and II, Euclidieae I and II, Chorisporeae, and Hesperideae, were similar, ranging from 13.1% to 20.4% (Table 4). Based on recent calculations for several Brassicaceae, corrected ITS distance values (Kimura two-parameter model) of 1% correspond to approximately 1 million years (Mya) (Koch & Al-Shehbaz, 2004). Estimated sequence divergence times for the above six clades were similar to those estimated among other tribal groups in the Brassicaceae (14 to 28 Mya; Lysak et al., 2005).

In conclusion, the ITS data proved a useful molecular tool to test the newly revised tribal limits

proposed for the Brassicaceae in Al-Shehbaz et al. (2006). In the present study, the ITS data supported the recognition of the tribes: Hesperideae (unigeneric, *Hesperis*) and Chorisporeae (3 genera, including *Chorispora*, *Diptychocarpus*, and *Parrya*), whereas the Anchonieae and the Euclidieae each separated into two distinct and distant clades (designated here as Anchonieae I and II and Euclidieae I and II). The data also supported the exclusion of eight genera from the latter four tribes, with appropriate tribal assignment given in parentheses: *Aubrieta* (Arabideae), *Blennodia* (Camelineae), *Erysimum* (Camelineae), *Goldbachia* (unresolved), *Hesperidanthus* (Schizopetaleae), *Notothlaspi* (unresolved), *Pseudocamelina* (Thlaspideae), and *Zuvanda* (unresolved).

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SYSTEMATIC REVISION AND PHYLOGENY OF *AINSLIAEA* DC. (ASTERACEAE, MUTISIEAE)[1]

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ABSTRACT

Ainsliaea DC. is an Asiatic genus of Mutisieae Cass. Forty-nine species and 19 varieties are recognized. Twenty-six species are endemic to China, four to Japan, four to Indo-China, two to the Himalayas, and one to Taiwan. The genus is characterized by its stems unbranched, with leaves basally rosulate or congested at the median part of the stem (only one species has stems branched, with alternate leaves), capitula few-flowered, florets deeply 5-cleft with unilateral lobes, and plumose pappus. Three new combinations are made: *Ainsliaea bonatii* Beauverd var. *multibracteata* (Mattf.) S. E. Freire, *A. glabra* Hemsl. var. *sutchuenensis* (Franch.) S. E. Freire, and *A. henryi* Diels var. *subalpina* (Hand.-Mazz.) S. E. Freire. One variety, *A. latifolia* (D. Don) Sch. Bip. var. *taiwanensis* S. E. Freire is described for the first time. The following taxa are newly synonymized: *A. macroclinidioides* Hayata var. *secundiflora* (Hayata) Kitam. and *A. secundiflora* Hayata with *A. apiculata* Sch. Bip. var. *acerifolia* Masam.; *A. aptera* DC. f. *paniculata* Kuntze with *A. aptera* DC.; *A. pteropoda* DC. var. *platyphylla* Franch. with *A. bonatii* Beauverd; *A. mattfeldiana* Hand.-Mazz. with *A. bonatii* var. *multibracteata*; *A. cleistogama* C. C. Chang with *A. cavaleriei* H. Lév.; *A. asarifolia* Hayata, *A. cordifolia* Franch. & Sav. var. *integrifolia* Maxim., *A. fragrans* Champ. ex Benth. var. *integrifolia* (Maxim.) Kitam., *A. integrifolia* (Maxim.) Makino, *A. ningpoensis* Matsuda, and *A. rubrifolia* Franch. with *A. fragrans* Champ. ex Benth.; *A. lancifolia* Franch. with *A. glabra* Hemsl.; *A. tenuicaulis* Mattf., *A. glabra* var. *tenuicaulis* (Mattf.) C. C. Chang, and *A. plantaginifolia* Mattf. with *A. glabra* Hemsl. var. *sutchuenensis* (Franch.) S. E. Freire; *A. angustifolia* Hook. f. & Thoms. ex C. B. Clarke with *A. glumacea* (Fr.) Sch. Bip.; *A. gracilis* Franch. var. *robusta* Diels with *A. grossedentata* Franch.; *A. morrisonicola* Hayata, *A. reflexa* Merr. var. *morrisonicola* (Hayata) Yamam., *A. henryi* Diels var. *ovatifolia* C. C. Chang, *A. reflexa* Merr. var. *nimborum* Hand.-Mazz., and *A. latifolia* (D. Don) Sch. Bip. var. *nimborum* (Hand.-Mazz.) Kitam. with *A. henryi* var. *subalpina*; *A. petelotii* Merr. with *A. hypoleuca* Diels ex H. Limpr.; *A. hui* Diels ex Mattf. with *A. kawakamii* Hayata; *A. heterantha* Hand.-Mazz. with *A. latifolia* (D. Don) Sch. Bip.; and *A. tonkinensis* Merr., *A. longipetiolata* Merr., and *A. reflexa* Merr var. *lobbiana* Beauverd with *A. reflexa* Merr. Lectotypes are newly designated for *Ainsliaea* DC., *Ainsliaea* sect. *Aggregatae* Beauverd, *A. apiculata* Sch. Bip., *A. aptera* DC., *A. cordifolia* Franch. & Sav., *A. crassifolia* C. C. Chang, *A. dentata* Koidz, *A. dissecta* Franch. & Sav., *A. elegans* Hemsl., *A. elegans* var. *strigosa* Mattf., *A. fulvipes* Jeffrey & W. W. Sm., *A. glabra* Hemsl., *A. hypoleuca* Diels ex H. Limpr., *A. macroclinidioides* Hayata, *A. multibracteata* Mattf., *A. ovalifolia* Vaniot, *A. pertyoides* Franch., *A. pertyoides* var. *albotomentosa* Beauverd, *A. pertyoides* var. *intermedia* Beauverd, *A. plantaginifolia* Mattf., *A. pteropoda* DC., *A. pteropoda* var. *macrocephala* Mattf., *A. pteropoda* var. *platyphylla* Franch., *A. pteropoda* var. *silhetensis* DC., *A. scabrida* Dunn, *A. tenuicaulis* Mattf., *A. yadismae* Koidz., and *Hieracium glumaceum* Fr. Neotypes are designated for *A. apiculata* Sch. Bip. var. *acerifolia* Masam., *A. apiculata* Sch. Bip. var. *ovatifolia* Masam., *A. cordifolia* Franch. & Sav. var. *integrifolia* Maxim., and *A. linearis* Makino. Epitypes are designated for *A. cordifolia* Franch. & Sav., *A. dissecta* Franch. & Sav., and *A. pteropoda* DC. Phylogenetic analysis of *Ainsliaea* based on 30 morphological characters produced three most parsimonious trees that support the monophyly of the genus. *Pertya* Sch. Bip., *Myripnois* Bunge, and *Macroclinidium* Maxim. are the genera closest to *Ainsliaea*, and it is hypothesized that *Pertya* is ancestral to the latter three. *Ainsliaea* is distinguished from *Pertya*, *Macroclinidium*, and *Myripnois* by its usually plumose pappus bristles (vs. scabrid pappus bristles in the other three genera). Phylogenetic analysis also supports the existence of two subgenera, i.e., *Ainsliaea* subg. *Ainsliaea*, with distinctly uniseriate pappus bristles, and the monospecific *Ainsliaea* subg. *Diaspananthus* (Miq.) Kitam. (*A. uniflora* Sch. Bip.) from Japan, characterized by its pappus of biseriate bristles. Phylogenetic resolution within subgenus *Ainsliaea* is weakly supported. However, the best supported resolution divides subgenus *Ainsliaea* into three subclades: two agree with two of three previously proposed sections, and the remaining basal third subclade is monospecific (*A. lancangensis* Y. Y. Qian). Two sections previously recognized, section *Ainsliaea* (= section *Scaposae* Beauverd including section *Frondosae* Beauverd) and section *Aggregatae* Beauverd, are tentatively proposed until more appropriate characters have been found. A key to the species of the genus is provided, as well as morphological descriptions, illustrations, and distribution maps.

Key words: *Ainsliaea*, Asia, Asteraceae, Compositae, Mutisieae, taxonomy.

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Ainsliaea DC. is an Asiatic genus of 49 species ranging from Afghanistan to Japan in warm temperate forests. The genus consists of three morphologically distinct assemblages: (1) one shrubby species; (2) 36 species of perennial herbs with leaves clustered at the base; and (3) 12 species, also perennial herbs but with leaves falsely verticillate at the lower half of the stem. Since the genus was proposed by de Candolle (1838), its only taxonomic treatment was carried out by Beauverd (1909) and included 33 species. Nevertheless, *Ainsliaea* was treated in regional works or floral treatments, such as Hooker (1881) for the Himalayas; Léveillé (1914, 1915), Institute of Beijing (1987), and Tseng (1996) for China; Kitamura (1965), Numata and Asano (1969), Hatusima (1975), Makino (1989), and Kitamura et al. (1998) for Japan; Ho (1993) for Vietnam; Hayata (1919) and Peng et al. (1998) for Taiwan; and Lee (1980) for Korea. These authors delimited *Ainsliaea* principally by its plumose pappus, few-flowered capitula, and florets deeply 5-cleft.

Ainsliaea differs from its most closely related genera, *Macroclinidium* Maxim., *Myripnois* Bunge, and *Pertya* Sch. Bip., in having plumose pappus bristles (vs. scabrid pappus bristles in the other three genera). All four genera were recognized by Panero and Funk (2002) as the tribe Pertyeae and subfamily Pertyoideae on the basis of molecular studies.

The present taxonomic treatment revises *Ainsliaea* considering morphological characters, habit, and distribution, with special reference to test the monophyly of the genus and to establish the relationships of its species. The present study also fully describes all species, evaluates their relationships, and provides, in several cases the first, illustrations of the species.

Taxonomic History

Ainsliaea was established by de Candolle (1838) as a new name for *Liatris* sensu D. Don (1825: 169) non *Liatris* Gaertn. ex Schreb., within the tribe Eupatorieae. De Candolle placed *Ainsliaea* in his "Mutisiaceae" (1838: 13), noting that this new genus and *Liatris* Gaertn. ex Schreb. have characters in common, e.g., rosulate habit, capitula homogamous, and pappus plumose, and *Ainsliaea* was distinguished by the anthers calcarate, caudate, with oblong apical appendages, and style branches shortly bilobed. De Candolle described two species and one variety, *A. pteropoda* and *A. aptera* both from Nepal, and *A. pteropoda* var. *silhetensis* from India. The only species described by D. Don under *Liatris*, i.e., *L. latifolia*, was referred by de Candolle as a synonym of *A. pteropoda* and later transferred to *Ainsliaea* by Schultz Bipontinus (1861: 190). The generic name honors Whitelaw Ainslie (1767–1836).

Botanists of different nationalities have contributed to the understanding of the genus. European botanical exploration in the 1800s led to the discovery of the first Chinese, Indo-Chinese, and Japanese species. Between 1852 and 1895, 22 species and six infraspecific taxa were described by British (*Ainsliea angustifolia* Hook. f. & Thoms. ex C. B. Clarke, *A. fragrans* Champ. ex Benth., *A. glabra* Hemsl., *A. ramosa* Hemsl., *A. walkeri* Hook. f.), German (*A. acerifolia* Sch. Bip., *A. apiculata* Sch. Bip., *A. aptera* DC. f. *paniculata* Kuntze, *A. asperrima* (D. Don) Sch. Bip., *A. brandisiana* Kurz, *A. glumacea* (Fr.) Sch. Bip., *A. uniflora* Sch. Bip.), French (*A. affinis* Miq., *A. apiculata* Sch. Bip. var. *minor* Miq., *A. cordifolia* Franch. & Sav., *A. dissecta* Franch. & Sav., *A. gracilis* Franch., *A. grossedentata* Franch., *A. lancifolia* Franch., *A. nervosa* Franch., *A. pertyoides* Franch., *A. pteropoda* DC. var. *leiophylla* Franch., *A. pteropoda* DC. var. *obovata* Franch., *A. pteropoda* DC. var. *platyphylla* Franch., *A. rubrifolia* Franch., *A. sutchuenensis* Franch., *A. yunnanensis* Franch.), and Russian (*A. cordifolia* Franch. & Sav. var. *integrifolia* Maxim.) botanists.

In the early twentieth century, seven species and one variety were added from China (*Ainsliaea henryi* Diels, *A. undulata* Diels, *A. gracilis* Franch. var. *robusta* Diels, *A. elegans* Hemsl., *A. ovalifolia* Vaniot, *A. sparsiflora* Vaniot, *A. spicata* Vaniot, and *A. scabrida* Dunn), one from the Philippines (*A. reflexa* Merr.), and one from Taiwan (*A. macroclinidioides* Hayata).

Beauverd (1909) described three new species and four varieties, *Ainsliaea faurieana* and *A. liukiuensis* from Japan, and *A. bonatii*, *A. bonatii* var. *glabra*, *A. bonatii* var. *arachnoidea*, *A. pertyoides* var. *albotomentosa*, and *A. pertyoides* var. *intermedia* from China. His treatment included 33 species. Based on plant habit, Beauverd established three sections in *Ainsliaea*: (1) *Frondosae*: *Ainsliaea pertyoides*, (2) *Scaposae*: *Ainsliaea angustifolia* (= *A. glumacea*), *A. aptera*, *A. asperrima*, *A. bonatii*, *A. brandisiana*, *A. elegans*, *A. fragrans*, *A. henryi*, *A. lancifolia* (= *A. glabra*), *A. pteropoda* (= *A. latifolia*), *A. ramosa*, *A. reflexa*, *A. rubrifolia* (= *A. fragrans*), *A. scabrida* (= *A. yunnanensis*), *A. spicata*, *A. sutchuenensis* (= *A. glabra* Hemsl. var. *sutchuenensis* (Franch) S. E. Freire), *A. undulata* (= *A. henryi*), *A. yunnanensis*, and (3) *Aggregatae*: *Ainsliaea acerifolia*, *A. apiculata*, *A. cordifolia*, *A. dissecta*, *A. faurieana* (= *A. linearis* Makino), *A. glabra*, *A. gracilis*, *A. grossedentata*, *A. hayatae* H. Lév. (= *A. henryi* Diels var. *subalpina* (Hand.-Mazz.) S. E. Freire), *A. integrifolia* (Maxim.) Makino (= *A. fragrans*), *A. liukiuensis* (= *A. apiculata* Sch. Bip. var. *acerifolia* Masam.), *A. nervosa*, *A. uniflora*, *A. walkeri*. Since Beauverd's monograph of

Ainsliaea, the number of valid names (excluding new names and new combinations) assigned to the genus has increased to 109, with 80 species and 29 infraespecific taxa recognized. Much of the increase may be attributed to taxa described by Chinese and Japanese botanists.

Kitamura (1938) transferred the monotypic genus *Diaspananthus* Miq. to *Ainsliaea*, and delimited two subgenera: (1) *Diaspananthus*, characterized by one-flowered capitula; and (2) *Eu-Ainsliaea* (= *Ainsliaea*) including two sections, sect. *Scaposae* and sect. *Aggregatae*.

Sugimoto (1957), in naming *Ainsliaea* × *hybrida*, was the only botanist to suggest hybridization in the genus.

Materials and Methods

HERBARIUM SPECIMENS

Loans of *Ainsliaea* from the following herbaria were examined: A, AAU, BKF, BM, CAL, CANB, CM, DAO, E, G, GH, HAST, HITBC, K, KUN, KYO, L, M, MAK, MICH, MO, NY, O, P, PE, PENN, PH, S, SMAO, TI, UC, US, W, WU (Holmgren et al., 1990; Appendices 1, 2). A list of specimens examined for the outgroup taxa is provided in Appendix 3. An index/list to scientific names mentioned in this article is provided in Appendix 4.

MORPHOLOGICAL ANALYSIS

Leaf trichomes were studied in samples cleared using the technique of Dizeo de Strittmatter (1973) and stained with 2% safranin. Descriptive terminology for the trichomes follows Ramayya (1962). Leaf descriptions were made utilizing the terminology of Harris and Harris (1994).

PHYLOGENETIC ANALYSIS

Characters. Twenty-nine morphological characters were used in the cladistic analysis; 14 characters are multistate and were treated as unordered. Twenty-four of the 29 characters were qualitative. Characters and their states used in the analyses are described in Table 1. Autapomorphies are not included unless they form one of the states in a multistate character. The data matrix is shown in Table 2.

Computer programs. Analyses using implied weights (Goloboff, 1993) were run in PeeWee ver. 3 (Goloboff, 1998). All analyses were run using the default settings amb- and poly= (polytomies allowed). The following search strategy was applied to all data sets: "hold 10000: rseed 0; hold/40; poly=: amb-: mult*500; max*; swap*." Five hundred subsearches were performed each constructing the initial tree with tree bisection and reconnection (TBR) and retaining a maximum of 40 trees in each replicate (hold/40, mult*500). The resulting trees were swapped using TBR swapping cutting two sister nodes simultaneously. The default option "ambs" retained only nodes that are supported by unambiguous optimizations, while poly= treats trees as polytomous. A medium concave function, k = 3, was used.

Jackknife frequency values (Farris et al., 1996) based on Wagner trees were calculated using the available instruction file NONA, the Jak-run file.

Ingroup and outgroup selection. The ingroup contains 39 species of the total 49 recognized for *Ainsliaea*. Taxa with similar characters have been entered as a single taxon in the data matrix (safe deletion rules, Wilkinson, 1995). Therefore, the following groups, *A. cavaleriei* H. Lév-*A. ramosa-A. rubrinervis* C. C. Chang, *A. bonatii-A. smithii* Mattf., *A. reflexa-A. henryi*, *A. glabra-A. nervosa*, *A. linearis-A. walkeri*, *A. brandisiana-A. spanocephala* Y. C. Tseng, are represented as *A. cavaleriei*, *A. bonatii*, *A. reflexa*, *A. glabra*, *A. linearis*, and *A. spanocephala*, respectively.

Ainsliaea crassifolia C. C. Chang, *A. nana* T. C. Tseng, and *A. paucicapitata* Hayata were omitted from analysis because they are only known from the original description and, consequently, too many characters are missing.

Pertya, *Myripnois*, and *Macroclinidium* were represented by *Pertya ovata* Maxim., *Myripnois dioica* Bunge, and *Macroclinidium robustum* Maxim., respectively, as outgroups in the present study because they share with *Ainsliaea* corollas deeply 5-lobed with unilateral lobes (unique character within the Mutisieae). *Macroclinidium*, which is endemic to Japan, may be regarded as the sister group of *Ainsliaea* due to its leaves congested at the median part of the stem and its herbaceous habit. *Pertya*, which is endemic to the Sino-Japanese region, is characterized by its shrubby habit and branched stems with leaves alternate (or nested on brachyblasts, which also occurs in the monotypic *Myripnois* from China) and was used for the purpose of rooting the trees in analysis. Characters delimiting the species of *Pertya* and *Macroclinidium* are autapomorphies and are therefore disregarded.

Results

MORPHOLOGICAL ANALYSIS

Habit. The differences in habit displayed by *Ainsliaea* species basically correlate with the infrageneric classification followed in this treatment.

Table 1. Morphological characters used in phylogenetic analysis of *Ainsliaea*.

Habit

1. Shrub or subshrub (0), perennial herb (1)
2. Stem branched (0), simple (1)

Leaves

3. Arrangement alternate (0), pseudoverticillate or clustered above stem base (1), as basal rosette (2), clustered from brachyblast (3)
4. Blade shape ovate (0), deltoid, ovate to deltoid or reniform (1), obovate or pandurate (2), elliptic or oblong to elliptic (3), lanceolate (4), linear, linear to elliptic, or linear to obovate (5)
5. Blade margin entire to obscurely denticulate (0), denticulate (1), dentate (2), palmatilobed or palmatifid (3), dissected (4)
6. Blade base rounded (0), attenuate (1), cuneate (2), cordate or cordate to sagittate (3)
7. Blade apex acute (0), acuminate (1), obtuse (2), rounded (3)
8. Texture chartaceous (0), coriaceous (1), membranous (2), slightly fleshy (3)
9. Leaf color slightly discolorous (0), distinctly discolored (1)
10. Abaxial blade surface strigose (0), glabrate to glabrous (1), sericeous (2), strigose to lanate or sparsely lanate (3)
11. Glandular trichomes absent (0), present (1)
12. Trichomes T-shaped (0), straight, type A (1), straight, type B (2), straight and triseriate (3), crisped (4)
13. Petiole length to 10 mm (0), more than 4 cm (1)
14. Petiole winged (0), wingless (1)
15. Petiole pubescence glabrate or scattered strigose (0), densely strigose (1)

Capitula

16. Arrangement solitary or 3 to 8 and all terminal (0), thyrsoid (1), stachyoid (2)
17. Peduncle ebracteate (0), bracteate (1)
18. Involucre 12–18 mm high (0), less than 10 mm high (1)
19. Phyllary 3- to 5-nerved (0), 1-nerved, with only midvein (1)
20. Phyllary apex acute (0), acuminate (1), obtuse or rounded (2)
21. Phyllary pubescence glabrous or glabrate (0), pilose (1), ciliate (2)

Florets

22. Floret number 5 (0), 6 or 7 or more (1), 3 or 4 (2), 1 or 2 (3)
23. Apical anther appendages apiculate or acute (0), rounded or truncate (1), emarginate (2)
24. Anther tails shortly pilose or pilose (0), smooth to slightly pilose (1)
25. Style branches truncate or rounded (0), acute or obtuse (1)
26. Pappus scabrose (0), plumose (1)
27. Pappus 65 to 100 bristles (0), 55 bristles (1), 30 to 40 bristles (2)
28. Pappus variable in length and width (0), similar in length and width (1)
29. Achene pubescence pilose (0), apically pilose (1), glabrous (2)

The genus contains 12 perennial herbs with leaves congested at midpoint (belonging to section *Aggregatae*) and 36 scapose perennial herbs with rosulate leaves or with leaves approximated above the base (most of them belonging to section *Ainsliaea*). There is only one shrub species, with leaves alternate on long branchlets in the upper part (*A. pertyoides*).

Pubescence. Pubescence in *Ainsliaea* species consists of six basic trichome types. The type of pubescence is often useful alone or in combination with other characters in distinguishing species and subspecies, e.g., *A. brandisiana*, *A. elegans*, *A. spanocephala*, *A. yunnanensis*. Six basic types are as follows (the first five present on stems, leaves, and phyllaries, and the sixth restricted to the achenes):

1. T-shaped, multistoried trichomes (Fig. 1A). This type of trichome generally varies from 2.5–4 mm long, consisting of a uniseriate pedestal of two or three somewhat squared cells and usually three or four long apical cells transversely elongated. The apical cells are shaped as in T-shaped trichomes, but with the distinction of having many cap cells. Considering the cellular development of the apical part, these trichomes can be slightly symmetrical (*Ainsliaea yunnanensis*) if the apical cells develop in two directions or asymmetrical (*A. macrocephala* (Mattf.) Y. C. Yseng, *A. mairei* H. Lév.) when the apical cells develop more in one direction than the other. Koyama (1975) referred to these trichomes as type B, occurring in some species of *Pertya*, e.g., *P. ovata*.

2. Long straight, uniseriate trichomes (Fig. 1B–E). This type of trichome generally varies from 0.7–3 mm long, consisting of a uniseriate pedestal of somewhat squared cells and long apical cells obliquely septate. Usually the apical part appears somewhat slanted due to horizontal growth exceeding

Table 2. Data matrix in the cladistic analysis. Polymorphic character states are scored as A = [01]; B = [02]; C = [03]; D = [12]; E = [14]; F = [23]; G = [24]; H = [013]. Data inapplicable to a taxon are represented by dashes.

Character	1 1234567890	2 1234567890	123456789
Pertya ovata	0000000000	000000-000	010000000
Myripnois dioica	0034010001	0-0-00-100	110010000
Macroclinidium robustum	1110221001	0111020012	010010000
Ainsliaea acerifolia	111-33-001	0211021002	020001212
A. angustata	1123010100	0111110100	120101210
A. apiculata	111-3CB000	0211021000	021001210
A. aptera	1121231000	11110D0000	121001210
A. apteroides	1120131003	1E11021010	021001210
A. bonatii	1121130003	1E10020110	121101210
A. brandisiana	1123032000	1311110101	100001210
A. caesia	1124000000	0101020100	021001212
A. cavaleriei	1120030000	0111110101	120001210
A. chapaensis	1120000100	1111020100	121001210
A. cordifolia	111-332000	0211021100	120111212
A. dissecta	111-43-201	0211021100	020111212
A. elegans	11210301AC	0E11110010	131001210
A. foliosa	111310000C	1G10020110	020001210
A. fragrans	1110030110	0111120101	022001211
A. fulvipes	1123002100	0111120110	121001210
A. glabra	1120121301	1211010110	02100121A
A. glumacea	1122110300	110--10111	021101210
A. gracilis	1110001000	0211021100	021001211
A. grossedentata	1110200000	0211021102	121011212
A. henryi	11200D0200	011A020111	1F100121D
A. hypoleuca	1120000013	0E10020111	121001210
A. kawakamii	11D0002001	0211010100	021001211
A. lancangensis	111-331000	1211010002	130001210
A. latifolia	112000000H	1E100D0110	121001210
A. linearis	1115110001	0210011102	0B2001210
A. macrocephala	1120000302	0010020011	121101110
A. macroclinidioides	111A231000	0211021100	021001210
A. mairei	1122010002	000--20110	121101210
A. nervosa	1125010101	1211010110	121001210
A. oblonga	1114D1A000	0211011100	021001210
A. parvifolia	1120120100	0111021100	021101211
A. pentaflora	1120020000	0111010010	101001210
A. pertyoides	000000110C	0E01000010	121001210
A. pingbianensis	1123002100	0111110101	120101210
A. qianiana	1123000301	0110020011	020001210
A. ramosa	1120030100	0111110101	020101210
A. reflexa	1120020201	011A020111	021001210
A. rubrinervis	1120030110	0111120101	020001210
A. smithii	11?1131003	1410010111	021101210
A. spanocephala	1123000100	1311110000	100001210
A. spicata	112200200C	0E01020110	021001210
A. trinervis	1114111001	0211010100	122001210
A. uniflora	112-33-000	0211010100	030011010
A. walkeri	1115110001	0?10011100	022001210
A. yunnanensis	1120120100	1011020010	021101210

the vertical. These trichomes are found in nearly all species of *Ainsliaea*. The cells of the apical part of these trichomes may be either slightly thicker (Fig. 1B–D; most species of section *Ainsliaea*) or thinner than those of the basal part (Fig. 1E; e.g., most species of *Ainsliaea* sect. *Aggregatae*). Koyama (1975) referred to these trichomes as type D and E, respectively, occurring in species of *Pertya*, e.g., *P.*

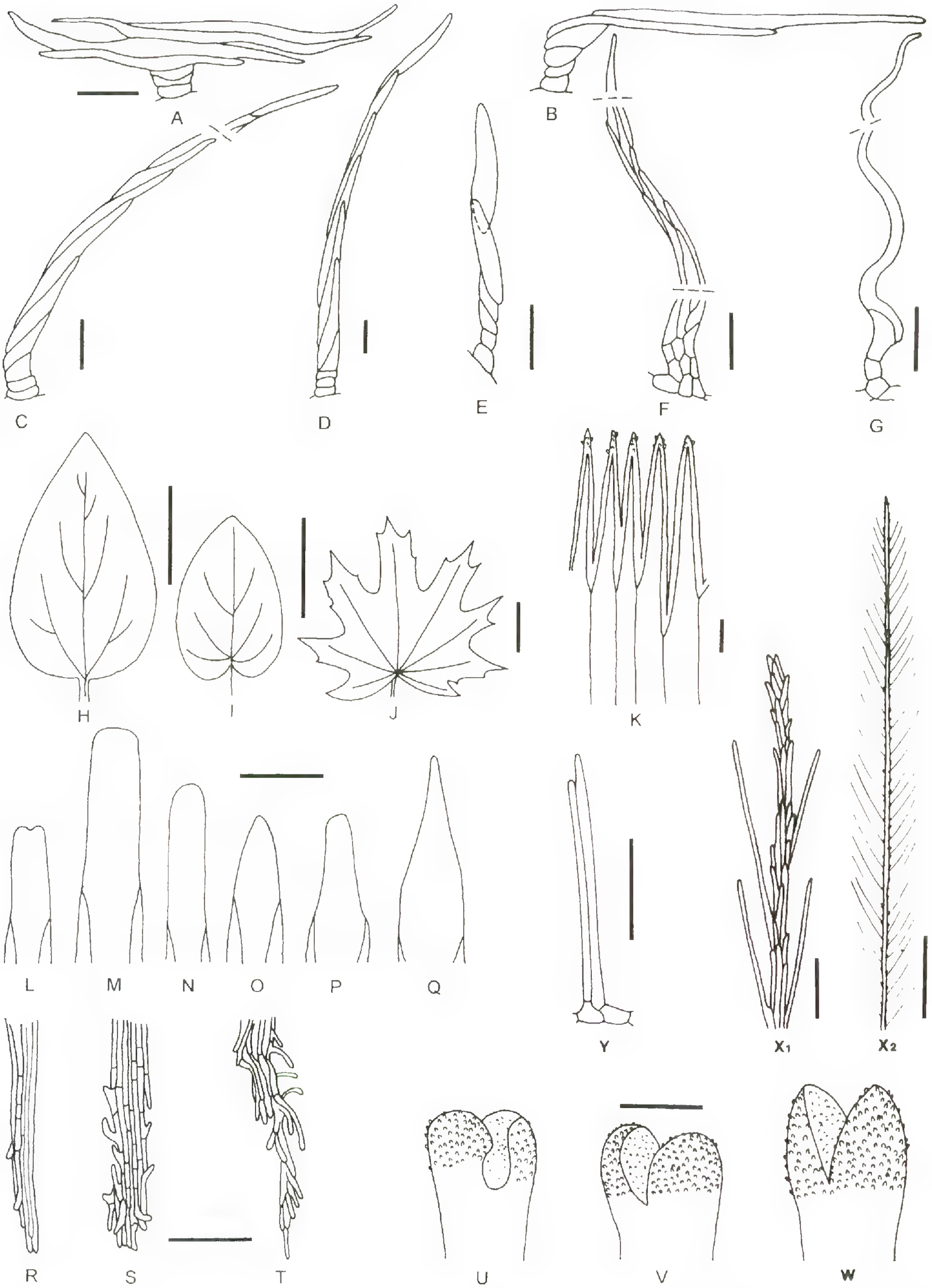


Figure 1. A–G. Foliar trichomes of *Ainsliaea*. —A. T-shaped trichome (*A. yunnanensis*, *Delavay 608*, NY). B–E. Straight trichomes. B–D: type A; E: type B (B, *A. chapaensis*, *Pételot 2068*, MO; C, *A. pertyoides*, *Delavay in 1888*, UC; D, *A. pingbianensis*, *S. Q. Chen 15094*, MO; E, *A. lancangensis*, *Y. Y. Qian 3343*, SMAO). —F. Straight multiseriate trichome (*A. brandisiana*, *Kurz 865*, G). –G. Crisped trichome (*A. bonatii*, *Ducloux 3646*, NY). H–J. Leaf venation of *Ainsliaea*. —H. Palmati-pinnate, 3-nerved (*A. latifolia*, *Biskam 43*, UC). —I. Palmati-pinnate, 5-nerved (*A. cavaleriei*, *Steward & Cheo 1090*, S). —J. Palmate (*A. acerifolia*, *Maximowicz in 1863*, MO). —K. Corolla venation of chasmogamous floret of *Ainsliaea* (*A. aptera*, *Maser 118*, US). L–Q. Apical anther appendages of *Ainsliaea*. —L. Emarginate (*A. walkeri*, *Ching 3216*, MO). –M, N. Rounded to truncate (M, *A. macrocephala*, *Handel-Mazzetti 3619*, W; N, *A. macroclinidioides*, *Tashiro in 1924*, KYO). —O. Acute (*A. foliosa*, HITBC 071881). —P, Q. Apiculate (P, *A. uniflora*, *Maximowicz in 1863*, S; Q, *A. cordifolia*, *Furuse in Oct 1956*, S). R–T. Anther tails of *Ainsliaea*. —R. Smooth to shortly pilose (*A. macrocephala*, *Handel-Mazzetti 3619*, W). —S.

hossei Craib. ex Hoss. (Koyama's type D with thick cells) and *P. glabrescens* Sch. Bip. (Koyama's type E with thin cells).

3. Long crisped trichomes (Fig. 1G). This type of trichome consists of one to three (occasionally five) basal cells and one long, twisted, and thin-walled, apical cell that is obliquely septate. They always occur intermixed with long straight trichomes, e.g., in *Ainsliaea hypoleuca* Diels ex H. Limpr., *A. pertyoides* var. *albotomentosa*, *A. spicata*, or with glandular trichomes, e.g., in *A. crassifolia*, *A. foliosa* Hand.-Mazz., *A. smithii*, or with both, e.g., in *A. apteroides* (C. C. Chang) Y. C. Tseng, *A. bonatii*, *A. latifolia*. Trichomes of this type were described by Ramayya (1962) as flagellate septate oblique, and they belong to type C according to Koyama (1975).

4. Long straight, multiseriate trichomes (Fig. 1F). This type of trichome generally varies from 0.7–0.8 mm long, consisting of a triseriate pedestal of square cells, and the apical portion also triseriate, of long cells obliquely septate. Trichomes of this type only occur in two species of *Ainsliaea* (*A. brandisiana* and *A. spanocephala* from Burma and Thailand, respectively).

5. Capitate and clavate-tipped glandular trichomes. These trichomes are found in the phyllaries, e.g., *Ainsliaea brandisiana*, *A. glumacea*, *A. nervosa*, *A. spanocephala*, and in leaves intermixed with other types of trichomes. These trichomes vary from clavate, biseriate, e.g., *A. aptera*, *A. foliosa*, *A. latifolia*, to conspicuously capitate, uniseriate (*A. apteroides*, *A. crassifolia*, *A. lancangensis* Y. Y. Qian, *A. yunnanensis*) or biseriate (*A. bonatii*, *A. chapaensis* Merr., *A. glabra*, *A. nervosa*, *A. smithii*).

6. Twin hairs or duplex hairs (Fig. 1Y). These trichomes are restricted to the achenes. These are found in nearly all species of *Ainsliaea* (except *A. acerifolia*, *A. caesia* Hand.-Mazz., *A. cordifolia*, *A. dissecta*, *A. henryi*, *A. gracilis*, *A. grossedentata*, and *A. nana*). They consist, in all species of *Ainsliaea*, of two small, square basal cells and two long apical cells acute at the apex, differing in length.

The cell structure of the long straight and T-shaped trichomes seems closely related, as was pointed out by Koyama (1975). In fact, this author suggested that the trichomes of *Ainsliaea yunnanensis* (T-shaped or type B) have the same pattern of those of *A. pertyoides* (long straight or type D), where the cells of the apical portion develop horizontally in two directions or in one direction, respectively. The long straight triseriate trichome may be considered to be a variant of the long straight uniseriate trichome.

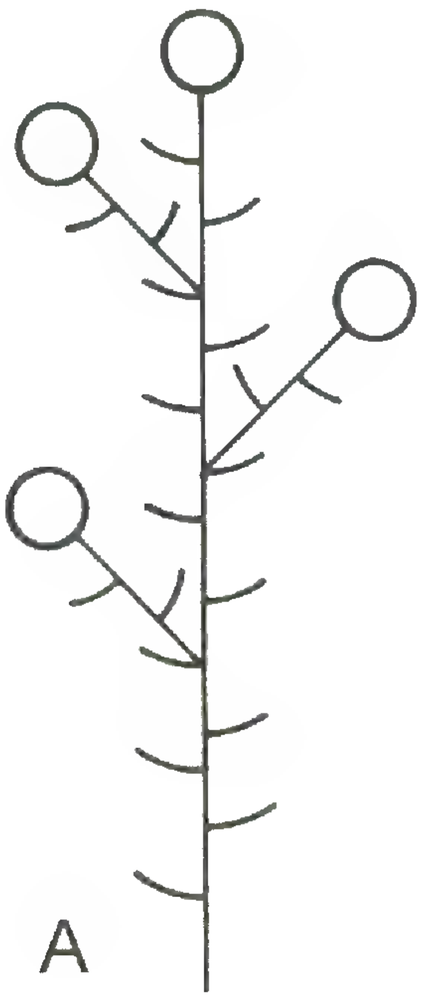
Leaves. Leaf characters are highly useful in distinguishing species in *Ainsliaea*. They are commonly long-petiolate (sessile in *A. glumacea* and *A. mairei* or short-petiolate in *A. caesia*, *A. pertyoides*, *A. spicata*), with petioles winged (*A. latifolia*, *A. bonatii*, *A. hypoleuca*) or wingless (*A. acerifolia*, *A. fragrans*, *A. lancangensis*, *A. pingbianensis* Y. C. Tseng). In most species of the genus, the leaf blades are entire (only *A. acerifolia*, *A. apiculata*, *A. cordifolia*, *A. dissecta*, *A. lancangensis*, and *A. uniflora* have dissected or lobed leaf blades), with margins denticulate-mucronate to dentate-mucronate. The leaf shape varies from ovate in the majority of species (*A. cavaleriei*, *A. elegans*, *A. fragrans*, *A. latifolia*) to elliptic (*A. angustata* C. C. Chang, *A. spanocephala*), linear (*A. linearis*), obovate (*A. spicata*), linear-obovate (*A. glumacea*), deltoid (*A. aptera*, *A. macroclinidioides*), or sub-rounded (*A. acerifolia*, *A. crassifolia*, *A. lancangensis*, *A. uniflora*). The base of leaf blades may be rounded (*A. chapaensis*, *A. pingbianensis*), cordate (*A. cordifolia*, *A. elegans*), or attenuate (*A. glabra*, *A. glumacea*).

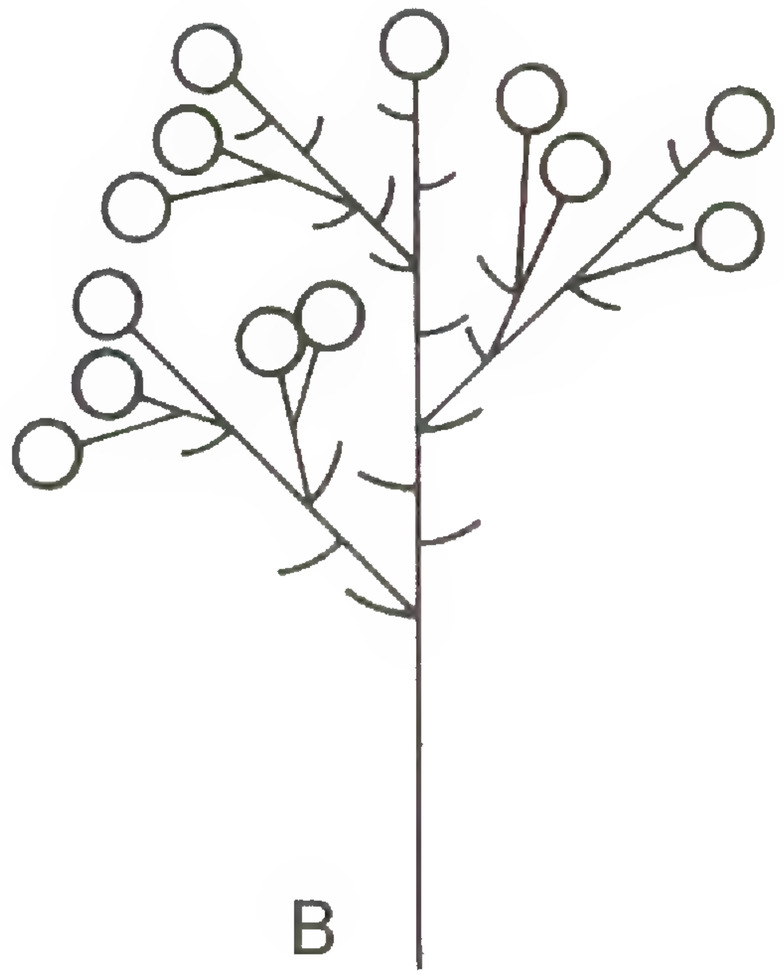
In most species of the genus, the venation is palmati-pinnate (Melville, 1976), 3- or 5-veined (Fig. 1H, I). This venation is intermediate between palmate and pinnate, with the distal part of the leaf pinnate and a basal pair (or two pairs) of major veins palmate. In the species with leaf blades palmatilobed or palmatiparted, the venation is palmate (rectipalmate, Melville, 1976; actinodromous, Hickey, 1973), with three, five, seven, or nine primary veins straight or nearly so, which radiate from a basal node (Fig. 1J).

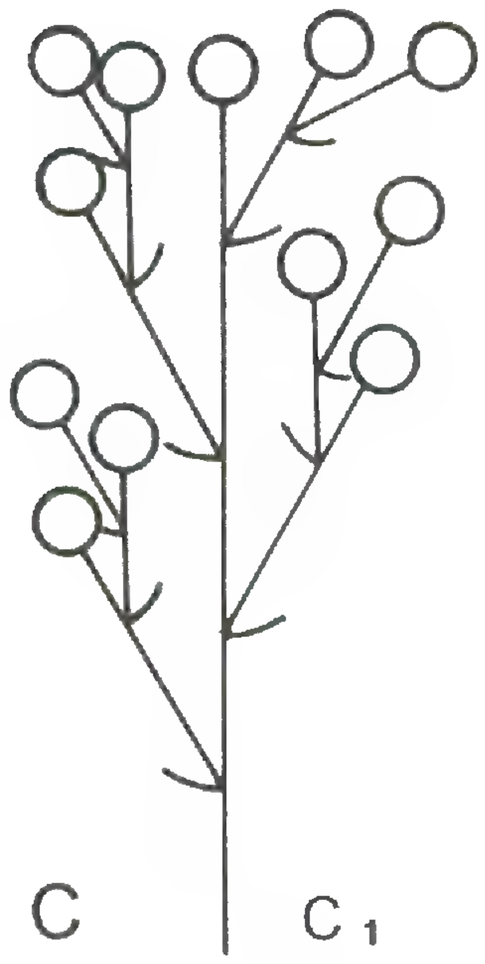
Synflorescence (Capitulescence). The synflorescence in *Ainsliaea* is monotelic (Weberling, 1985). The axis of the synflorescence ends in a terminal capitulum, and lower floral branches (paracladia) also end in terminal capitula. Thyrsoid and stachyoid synflorescences (Troll, 1964–1969; Weberling, 1985; Rua, 1999) prevail by far (Fig. 2).

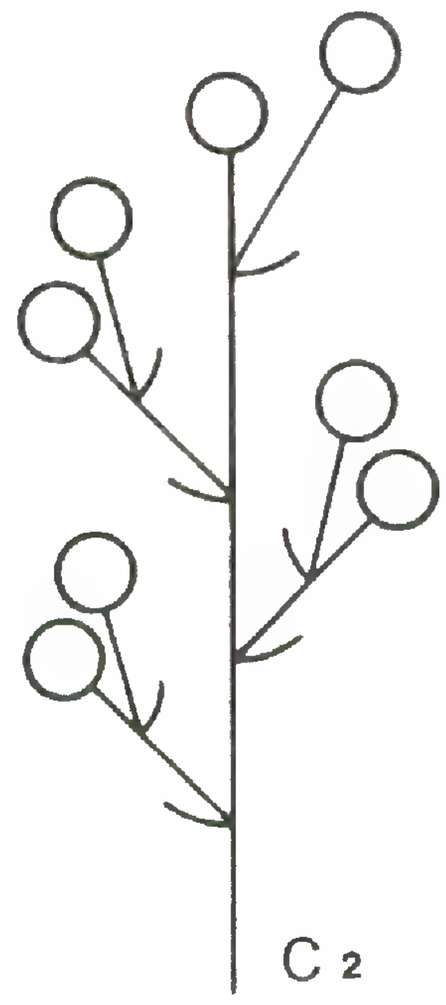
←

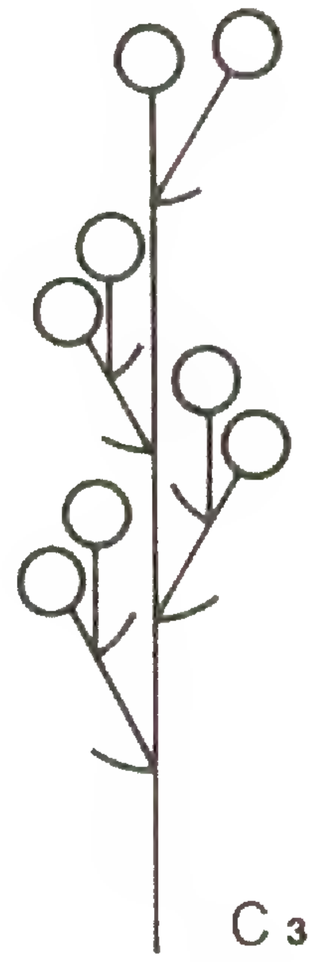
Shortly pilose (*A. fragrans, Tsang 20812*, S). —T. Pilose (*A. uniflora, Maximowicz in 1863*, S). U–W. Style branches of *Ainsliaea*. —U. Truncate (*A. elegans, Henry 10891*, MO). —V. Rounded (*A. aptera, Maser 118*, US). —W. Obtuse to acute (*A. cordifolia, Furuse in Oct 1956*, S). X_1–X_2. Pappus of *Ainsliaea* (*A. gracilis, Farges 1034A*, P). —X_1. Upper part of the bristle pappus. —X_2. Bristle pappus. —Y. Achene trichome (*A. macroclinidioides, Tashiro in 1924*, KYO). Scale bars: A–D, F, X_1, Y = 0.1 mm; E, G = 0.05 mm; H–J = 3 cm; K = 1 mm; L–W = 0.5 mm; X_2 = 1.5 mm.

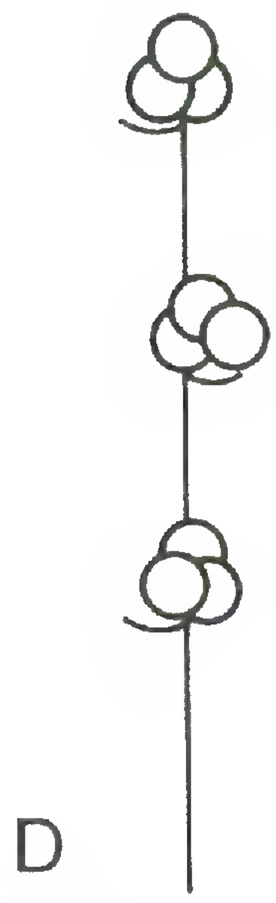












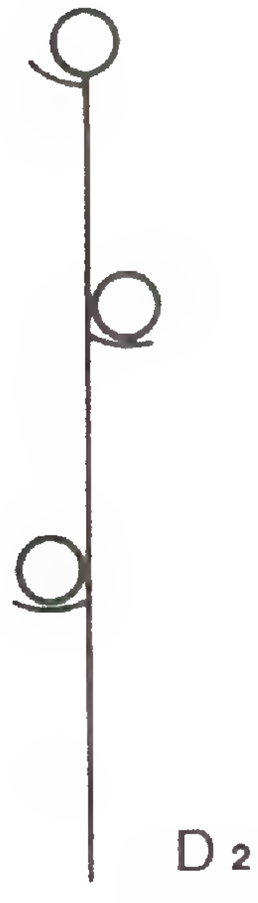


Figure 2. Synflorescences of *Pertya* and *Ainsliaea*. —A. Monotelic botryoid with leafy paracladia (*Pertya*). —B. Monotelic thyrsoid with leafy paracladia (*A. pertyoides*). —C. Monotelic thyrsoid with monochasial paracladia (C_1. *A. angustata*, *A. cavaleriei*, *A. elegans*, *A. glabra*, *A. glumacea*, *A. lancangensis*, *A. pingbianensis*, *A. ramosa*, *A. uniflora*, *A. smithii*. C_2. *A. brandisiana*, *A. pentaflora*, *A. spanocephala*. C_3. *A. linearis*, *A. nervosa*, *A. oblonga*, *A. trinervis*, *A. walkeri*). —D. Monotelic stachyoid (D_1. Capitula 3- to 5-fasciculate (three capitula shown): *A. acerifolia*, *A. apiculata*, *A. aptera*, *A. bonatii*, *A.*

The closely related genus *Pertya* also has monotelic synflorescences in which lateral and leafy floral shoots are produced from the axils of foliage leaves (Fig. 2A). Thus, these lateral floral shoots, present in *Pertya*, could be interpreted as the first step of a transition (the increase in number and the reduction of foliage leaves into bracts) to the branched system in *Ainsliaea*. In this view, the synflorescence of *A. pertyoides* (Fig. 2B), with leafy paracladia and capitula situated in the axils of foliage leaves, is regarded as the most primitive form of the synflorescence in *Ainsliaea*.

In *Ainsliaea* the main axis and lower paracladia have monochasial structure (Fig. 2C). By reduction of the capitula-bearing axis and its ramification, stachyoid inflorescences (cauliflory) occur (Fig. 2D).

Capitula. The capitula arranged laterally on the branches are peculiar in the species of the genus. Furthermore, at anthesis, the capitula with chasmogamous florets (*Ainsliaea acerifolia*, *A. cordifolia*, *A. dissecta*, *A. macrocephala*) are usually nodding. The adaptative significance of this morphology could be, following Sklenář (1999) who studied nodding capitula in Asteraceae, to protect the flowers from snow and rain as well as to attract insect pollinators due to temperatures warmer than other parts of the plant and the surrounding air.

Florets. In the genus *Ainsliaea*, florets on a capitulum are all hermaphroditic and isomorphic. They may be chasmogamous or cleistogamous. In fact, plants of *Ainsliaea* usually display a type of capitular dimorphism producing open heads consisting of chasmogamous florets as well as capitula of cleistogamous florets with involucres that remain completely closed until the mature achenes are released. Cleistogamous florets in *Ainsliaea* appear structurally modified for autogamy, i.e., the floret never opens (obligately self-pollinated), with the corolla tubular to pseudoligulate (one deeper slit but without expanded limb), stamens and styles much reduced in size, and fertile achenes larger than chasmogamous ones. Chasmogamous florets are adapted to xenogamy, i.e., the floret opens (to potentially cross-pollinate), with corollas ligulate (one deeper slit and lobes unilateral and reflexed), stamens and styles developed, and smaller, sometimes flat, and sterile achenes. These two types of florets may occur simultaneously on one individual (in some specimens of *A. fragrans*, *A. reflexa*) or occur separate in time (*A. pingbianensis*), i.e., chasmogamous florets are produced in warm months (usually May to August) and cleistogamous florets in colder months (usually September to January) (pers. obs.). In contrast, a few species such as *A. cavaleriei* and *A. elegans* have florets always cleistogamous or only chasmogamous, respectively, in warm as well cold seasons.

Chaptalia Vent. and *Leibnitzia* Cass. are two other genera of Asteraceae from which cleistogamous capitula have been reported (Burkart, 1944; Vuilleumier, 1969; Nesom, 1983). From an ecological perspective, cleistogamy is regarded as a dimorphic strategy (Lloyd, 1984; Schoen & Lloyd, 1984; Plitmann, 1995) where cleistogamous flowering is favored under adverse and stressful conditions when pollinators and resources are likely to be scarce (Schemske, 1978; Lord, 1981; Campbell et al., 1983).

Corollas. In all species of *Ainsliaea*, the corollas are white or pink, glabrous and papillose only in the upper part of the lobes. Venation in all tubular-pseudoligulate and ligulate corollas corresponds to a discoid type (Koch, 1930a, b) in which there are five vascular traces at the base (Fig. 1K). Each of these is formed by the fusion of adjacent marginal bundles of the connate petals. The traces run upward through the corolla and separate into constituent branches, just below the sinuses of the corolla lobes. The marginal bundles of each lobe unite at its apex. In *Ainsliaea*, five fused lateral bundles divide at different levels. resulting in lobes unequal in length. One of the slits is much longer than the others. Similar corolla venation is present in the genus *Cnicus* L. of the tribe Cardueae (Manilal, 1971).

Anthers. In species with chasmogamous florets, the anthers are exserted and usually purple. In species with cleistogamous florets, the anthers are shorter than the corolla. Anthers in *Ainsliaea* have basal tails and long apical appendages. The tails can be smooth to slightly pilose (Fig. 1R; *Ainsliaea cordifolia*, *A. macrocephala*) or more commonly short-pilose (Fig. 1S; *A. apiculata*, *A. fragrans*, *A. reflexa*, *A.*

←

caesia, *A. crassifolia*, *A. dissecta*, *A. fulvipes*, *A. foliosa*, *A. henryi*, *A. hypoleuca*, *A. latifolia*, *A. macroclinidioides*, *A. macrocephala*, *A. mairei*, *A. qianiana*, *A. reflexa*, *A. spicata*, *A. yunnanensis*. D_2. Capitula 1- to 3-fasciculate (only one capitulum shown): *A. apteroides*, *A. chapaensis*, *A. cordifolia*, *A. fragrans*, *A. gracilis*, *A. grossedentata*, *A. kawakamii*, *A. parvifolia*, *A. paucicapitata*, *A. rubrinervis*).

pertyoides); rarely they are pilose (Fig. 1T; *A. uniflora*). The feature of apiculate anther appendages that Bremer (1994) considered to be a synapomorphy for *Ainsliaea* is variable within the genus; most species have apical anther appendages rounded or truncate (Fig. 1M, N), some have apiculate appendages (Fig. 1P, Q), and a few may be emarginate (Fig. 1L; *A. walkeri*, *A. linearis*) or acute (Fig. 1O; *A. foliosa*). Furthermore, apiculate apical appendages also appear in species apparently not related from a morphological point of view (*A. uniflora*, *A. angustata*, *A. dissecta*).

Style branches. In the style of all species of *Ainsliaea*, there are two vascular bundles. This has been described previously by Carlquist (1957) in *Stifftia* J. C. Mikan, *Stenopadus* S. F. Blake, and *Stomatochaeta* (S. F. Blake) Maguire & Wurdack from Mutisieae in Guayana. The style branches in all species of *Ainsliaea* are bilobate or shortly bifid (eventually longer in cleistogamous florets), and dorsally short-pilose. The apex is commonly truncate (Fig. 1U; *A. apiculata*, *A. glabra*, *A. lancangensis*, *A. reflexa*, *A. yunnanensis*), may be merely rounded (Fig. 1V; *Ainsliaea aptera*, *A. hypoleuca*, *A. linearis*), or, more rarely, obtuse to acute (Fig. 1W; *A. cordifolia*).

Pappus. The pappus in *Ainsliaea* consists of pilose and rigid bristles of all the same length and width, with bristle margins scabrid to plumose (Fig. 1X). The pappus is uniseriate in most species, with usually 30 to 40 bristles, and only *A. uniflora* exhibits a biseriate pappus, with 65 to 70 bristles. In *A. macrocephala*, with ca. 55 bristles, an intermediate condition occurs. The pappus of cleistogamous florets exceeds the corolla in length, whereas the pappus of chasmogamous florets is shorter than their associated corollas.

Achenes. In the achenes of *Ainsliaea* usually 10 (rarely nine or 11) vascular bundles are present. In all these achenes, these bundles fuse into a single vascular strand at the achene base. The presence of 10 (or nine) bundles in achenes of Mutisieae has been described previously by Carlquist from Guayana Mutisieae (1957), in *Wunderlichia* Riedel ex Benth. & Hook. f., and *Hesperomannia* A. Gray (Carlquist, 1976).

Pollen. Pollen morphology in *Ainsliaea* has been studied for 13 species (Marticorena & Parra, 1975; Tellería & Katinas, 2005). The pollen of *Ainsliaea* is commonly tricolpate, ocasionally tricolporate, and microechinate, with ectosexina and endosexina poorly differentiated and compact. According to Tellería and Katinas (2005), this compact structure with poorly differentiated sublayers represents a unique exine type within the Mutisieae s.l.

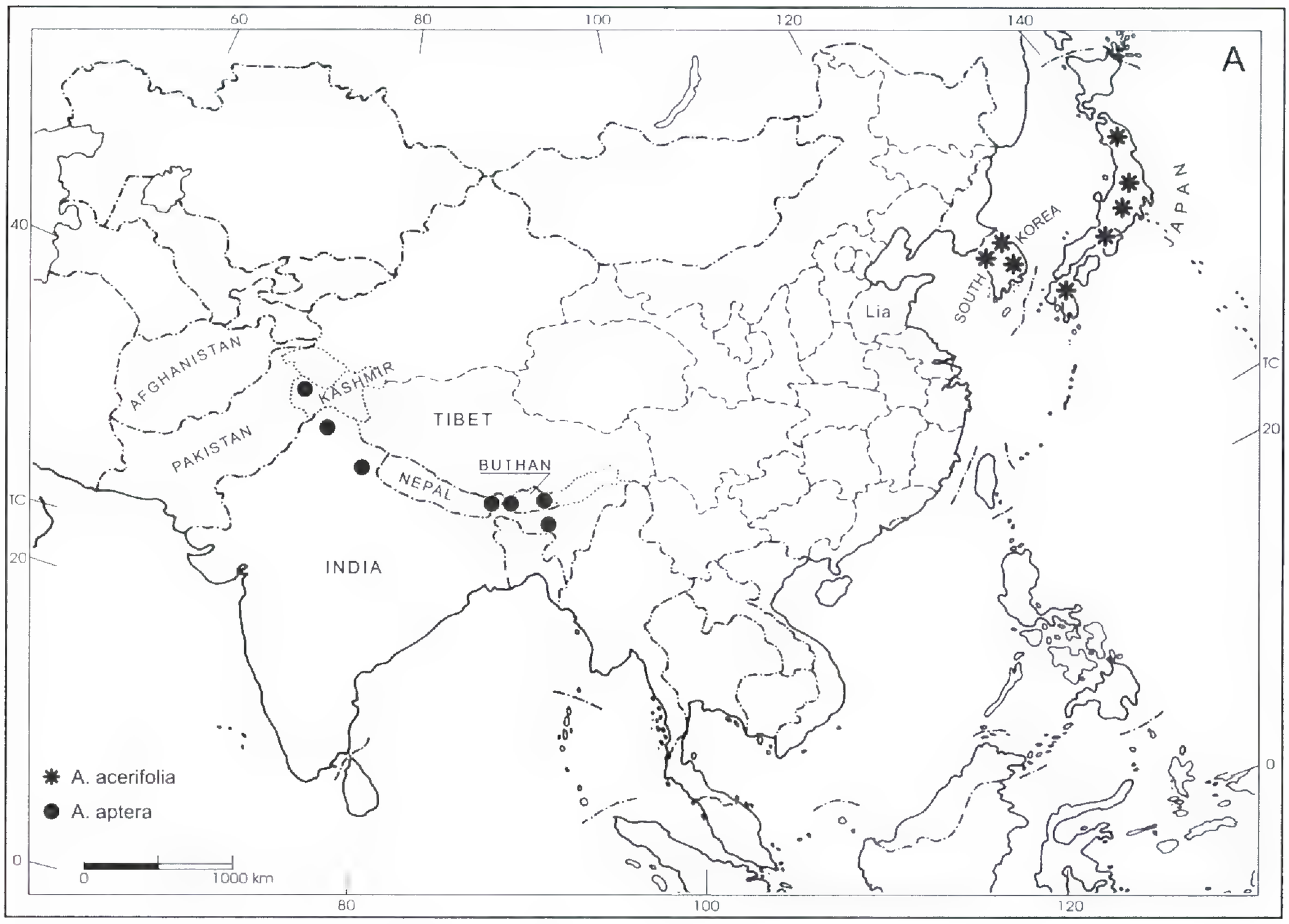
Chromosome number. Arano (1965) suggested that *Ainsliaea* and *Pertya* may be considered phylogenetically closely related from the karyological perspective. This author pointed out that the basic number $n = 13$ in *Ainsliaea* (*A. acerifolia*, *A. apiculata*, *A. dissecta*, *A. fragrans*, *A. uniflora*, Arano 1963; *A. faurieana* × *apiculata* [= *A.* × *hybrida*], *A. linearis*, Watanabe et al., 1992) seems to have been derived from $n = 14$ in *Pertya* by aneuploid reduction. In this sense, the haploid number $n = 12$, more recently reported for two species in *Ainsliaea*, *A. aptera* (Mehra et al., 1965; Gupta et al., 1989) and *A. latifolia* (Malla et al., 1977; Peng & Hsu, 1977, 1978), also appears to be aneuploid derived from $n = 13$.

Conversely, the more symmetrical karyotype of *Ainsliaea*, with respect to that of *Pertya*, is considered by Arano (1965) to be a relictual character in *Ainsliaea*.

DISTRIBUTION AND ECOLOGY

Ainsliaea is an Asiatic genus occurring from Afghanistan to Japan (Figs. 3–7). *Pertya*, with ca. 20 species and putatively ancestral to *Ainsliaea*, *Myripnois*, and *Macroclinidium*, shows a similar distribution pattern from Afghanistan, China, Thailand, and Japan. *Myripnois* is endemic to China (Beijing), and ca. six species of *Macroclinidium* are found in Japan.

Although five species of *Ainsliaea* are known only from their type locality (*A. caesia*, *A. chapaensis*, *A. nana*, *A. parvifolia* Merr., *A. paucicapitata*), China evidently represents the center of species distribution. There are 37 species in China, 26 of which are endemic to that country, especially well represented in Yunnan (20 species), Sichuan (18), and Guangdong (14). Two of these 36 (*A. fragrans* and *A. oblonga* Koidz.) extend their ranges to Japan (Figs. 4, 6), three (*A. elegans*, *A. hypoleuca*, and *A. reflexa*) extend to Southeast Asia and Indonesia (Figs. 4, 5, 7), and two (*A. latifolia* and *A. spicata*) extend here, and west to the Himalayas (Figs. 5, 7). Two species, *A. aptera* and *A. glumacea*, are restricted to the Himalayas (Figs. 3, 5). Four species (*A. brandisiana*, *A. chapaensis*, *A. pentaflora* S. E. Freire, *A. spanocephala*) occur only in Southeast Asia (Fig. 3). Four species (*A. cordifolia*, *A. dissecta*, *A. linearis*, *A. uniflora*) are endemic to Japan (Figs. 4, 5, 7). Two species, *A. acerifolia* and *A.*



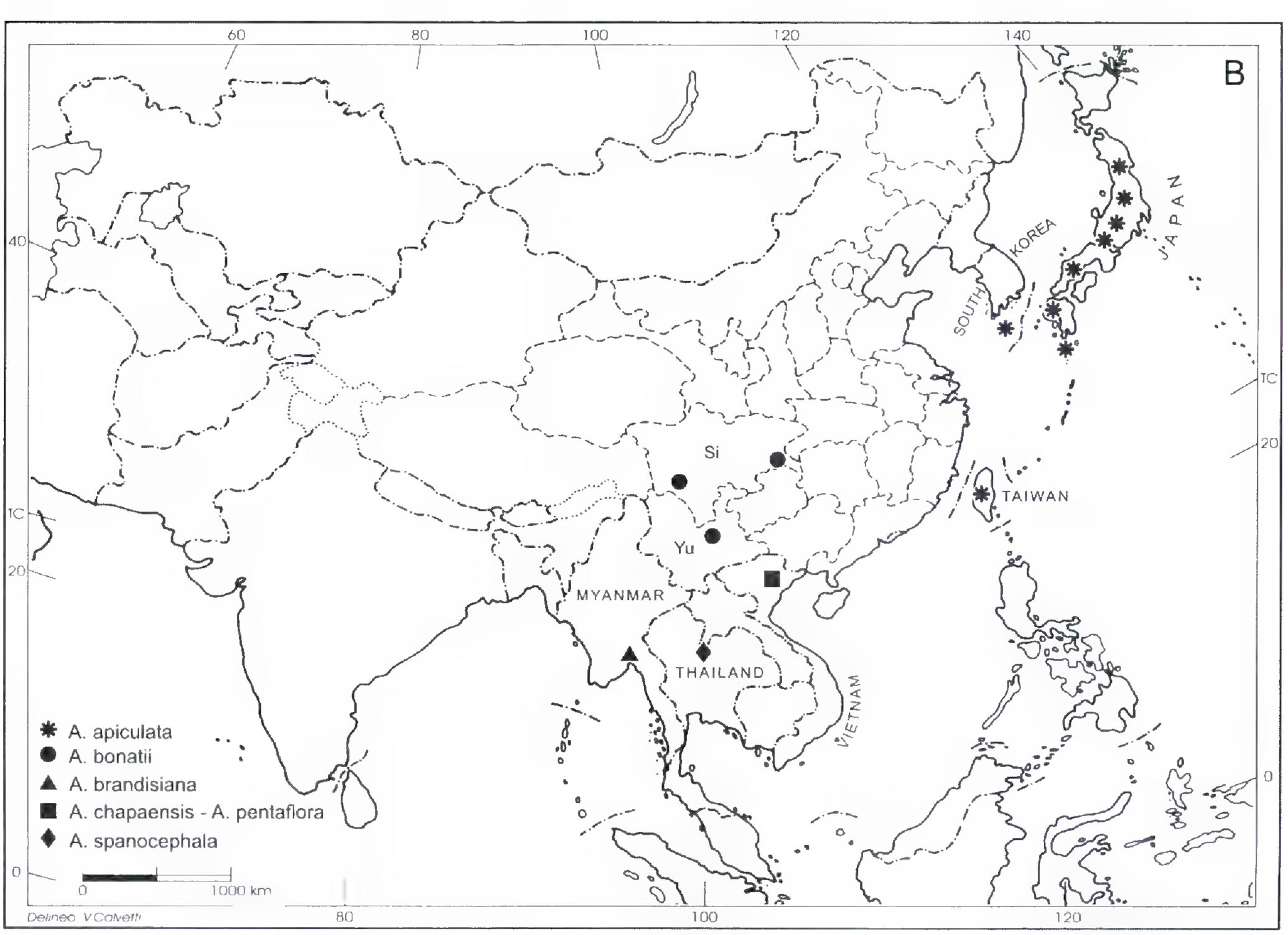
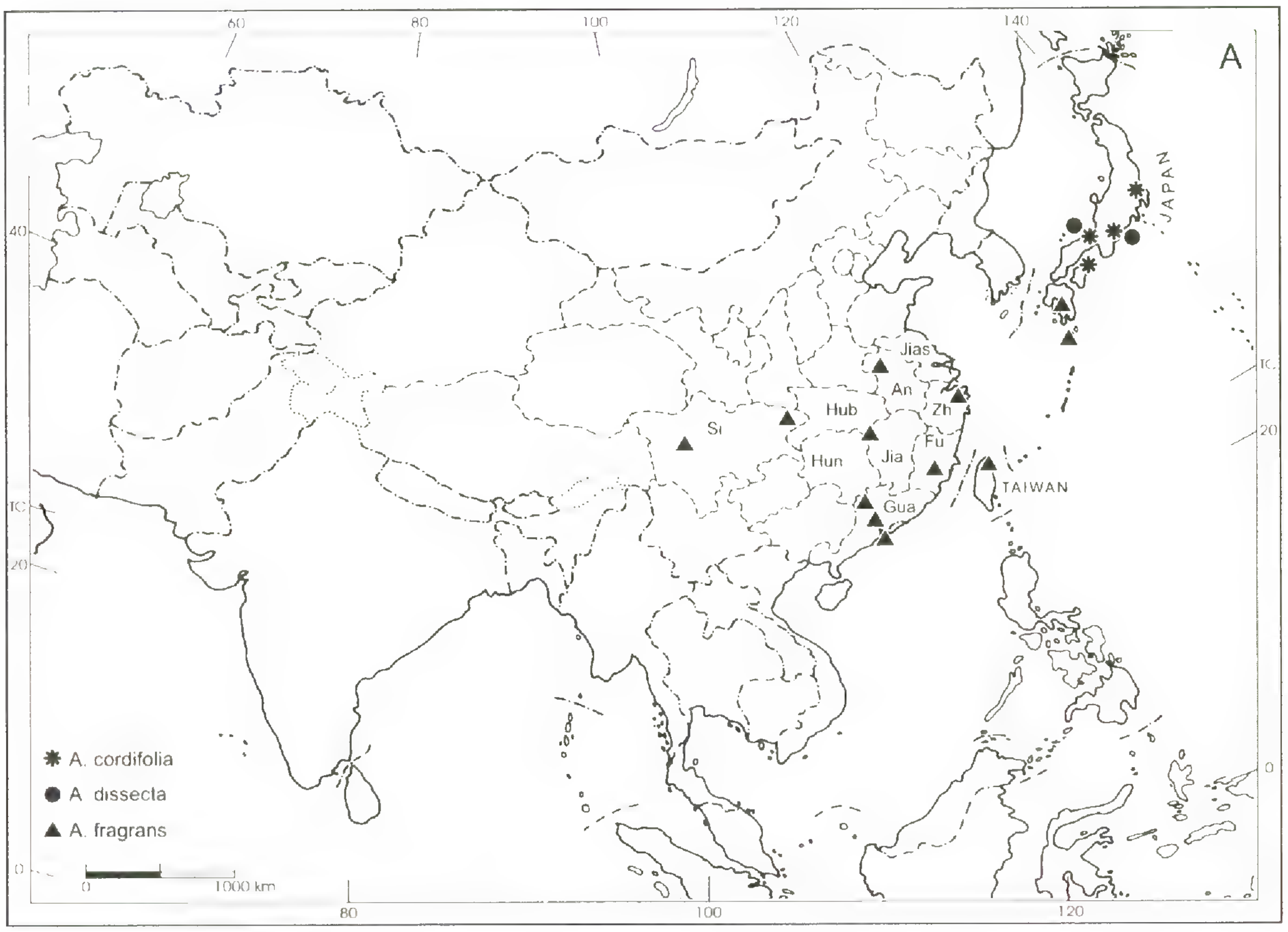


Figure 3. A. Distributions of *Ainsliaea acerifolia* (locality within Liaoning (China) unknown) and *A. aptera* (localities within Afghanistan, Nepal, Pakistan, and Tibet unknown). —B. Distributions of *Ainsliaea apiculata*, *A. bonatii*, *A. brandisiana*, *A. chapaensis*, *A. pentaflora*, and *A. spanocephala*. Lia = Liaoning; Si = Sichuan; Yu = Yunnan.



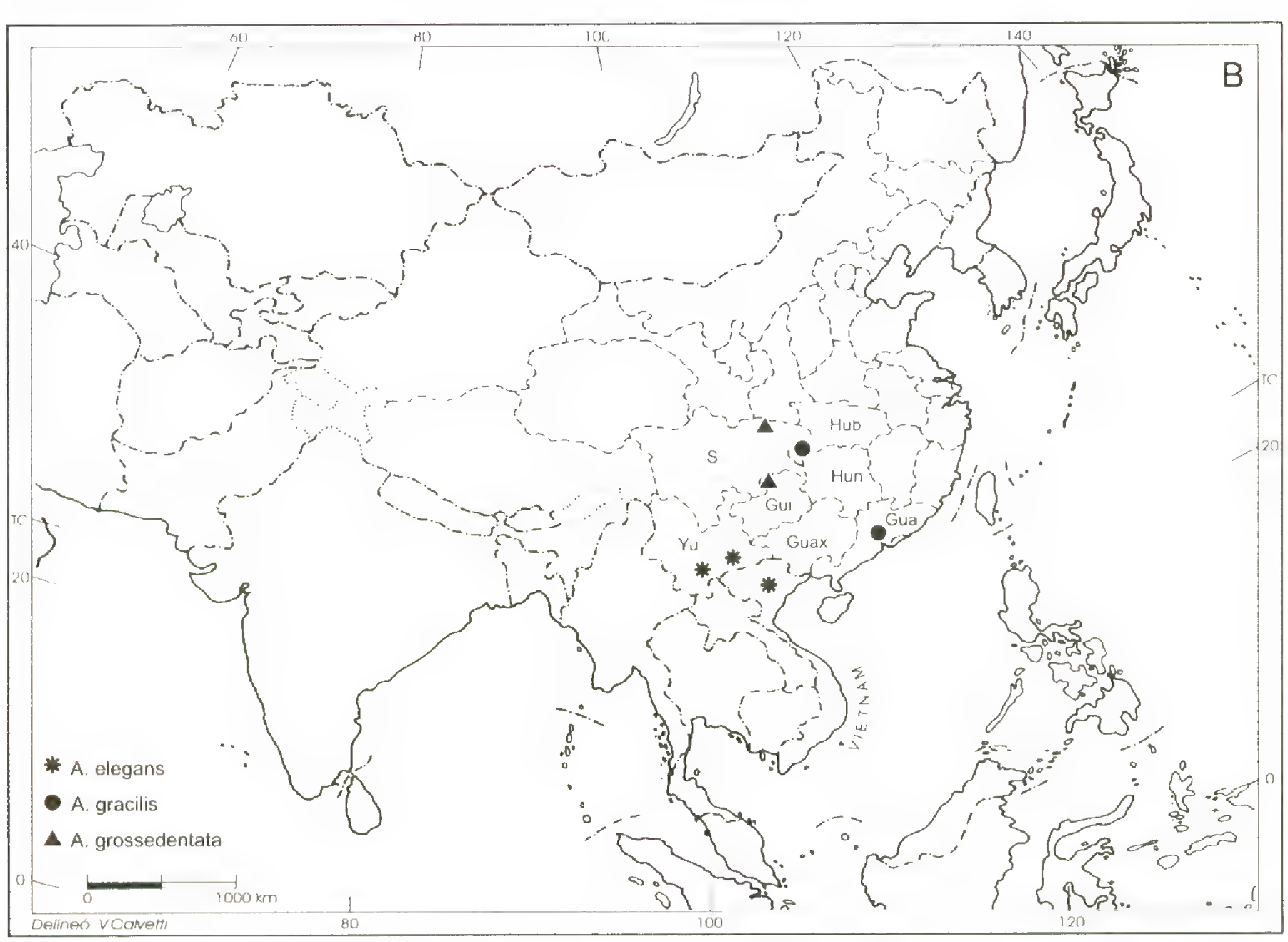
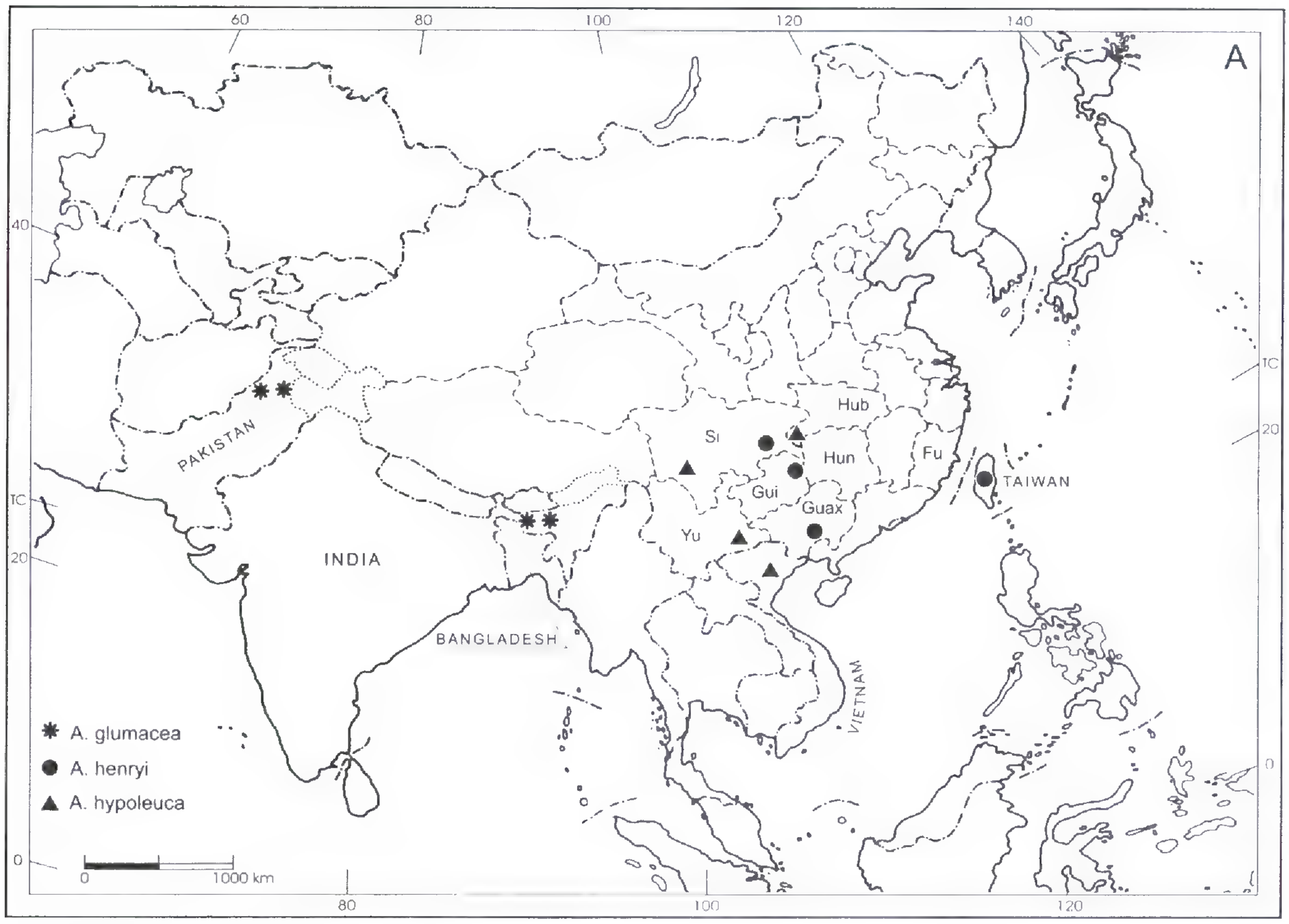


Figure 4. A. Distributions of *Ainsliaea cordifolia*, *A. dissecta*, and *A. fragrans* (localities within Hubei, Hunan, and Jiangsu unknown). —B. Distributions of *Ainsliaea elegans* (locality within Guizhou unknown), *A. gracilis* (localities within Guangxi and Hunan unknown), and *A. grossedentata* (locality within Hubei unknown). An = Anhui; Fu = Fujian; Gui = Guizhou; Gua = Guangdong; Guax = Guangxi; Hub = Hubei; Hun = Hunan; Jias = Jiangsu; Jia = Jiangxi; Si = Sichuan; Yu = Yunnan; Zh = Zhejiang.



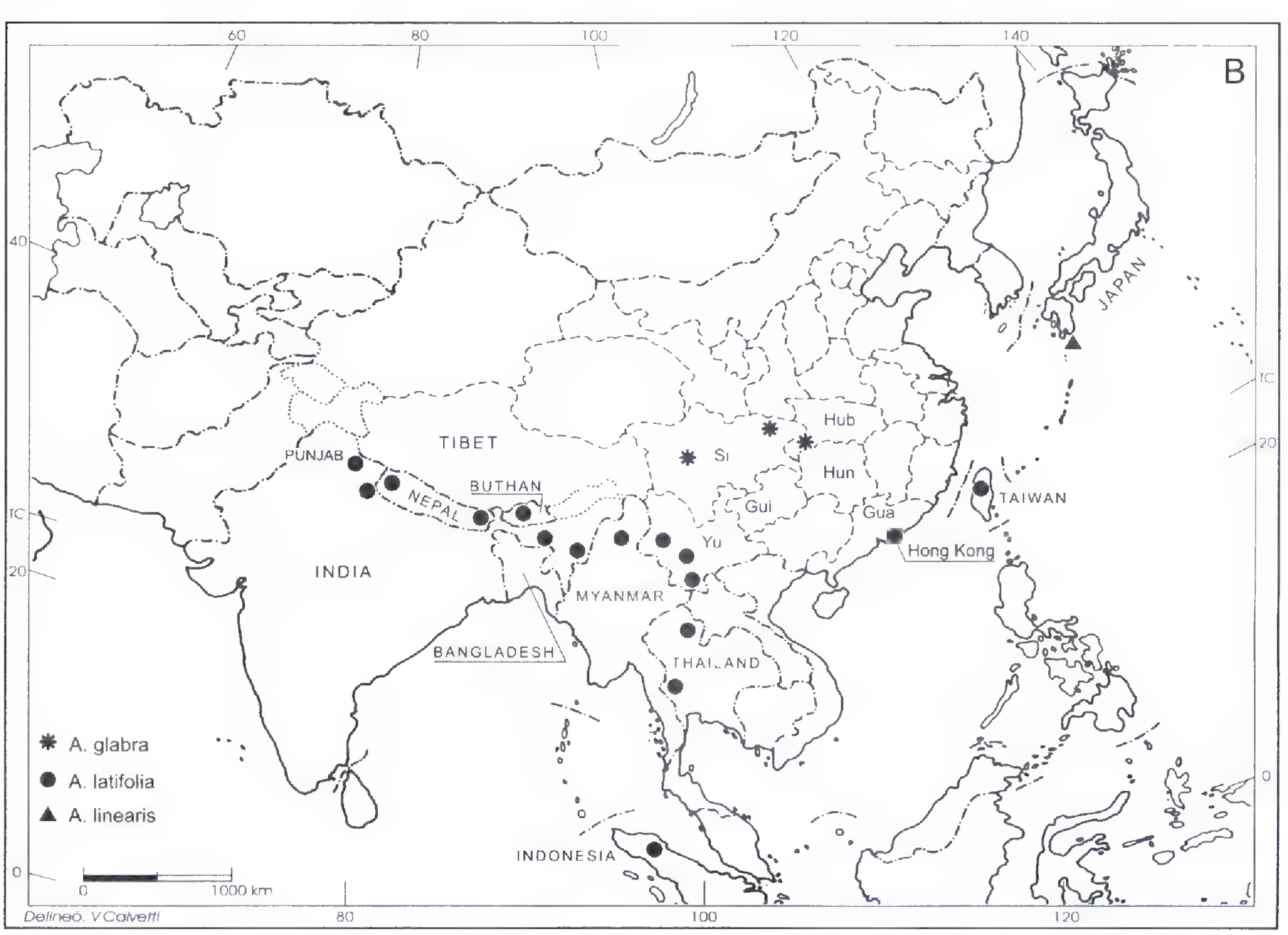
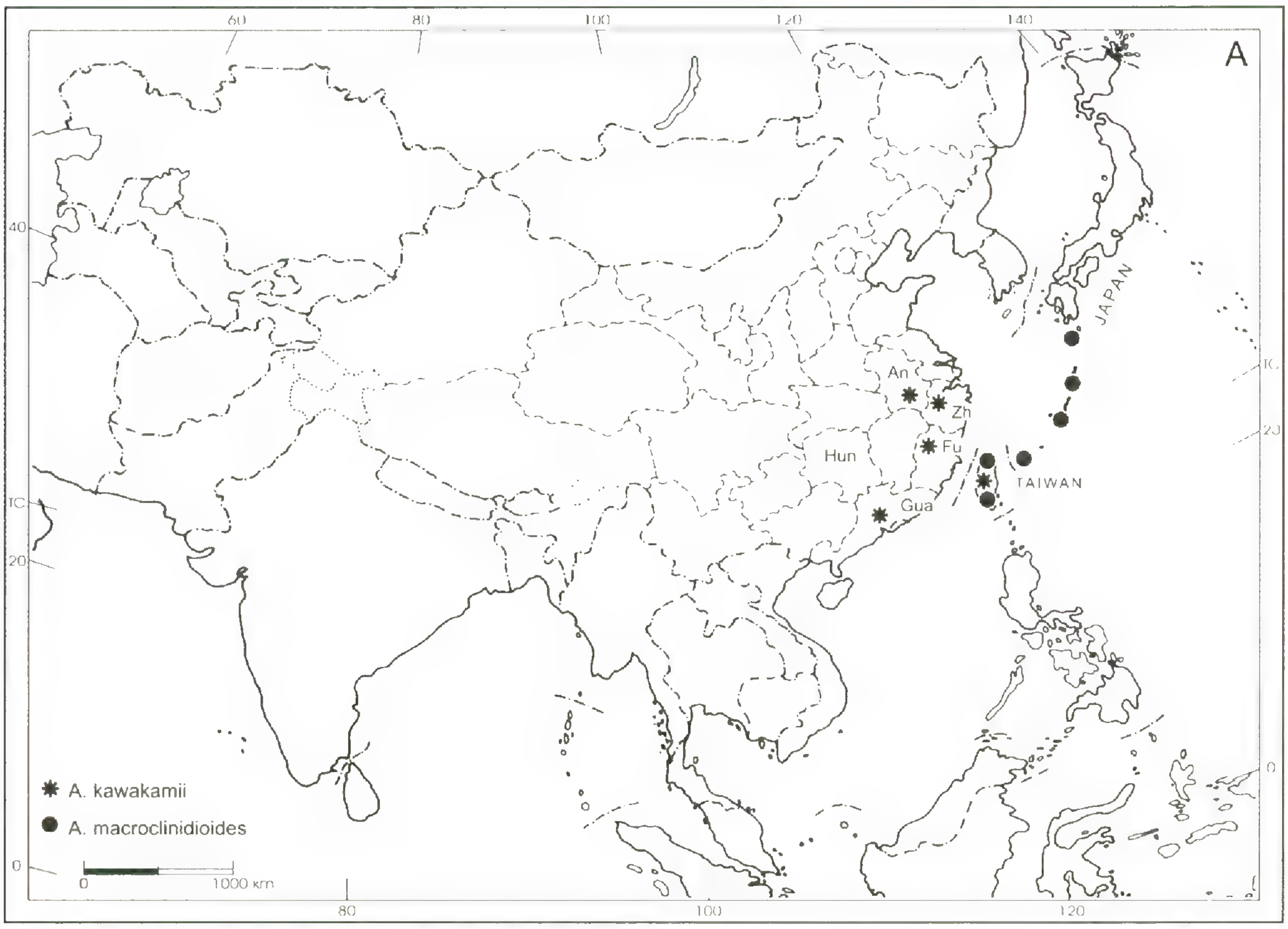


Figure 5. A. Distributions of *Ainsliaea glumacea*, *A. henryi* (localities within Fujian, Hubei, Hunan, and Yunnan unknown), and *A. hypoleuca*. —B. Distributions of *Ainsliaea glabra* (localities within Guizhou, Hunan, Tibet, and Yunnan unknown), *A. latifolia*, and *A. linearis*. Fu = Fujian; Gui = Guizhou; Gua = Guangdong; Guax = Guangxi; Hub = Hubei; Hun = Hunan; Si = Sichuan; Yu = Yunnan.



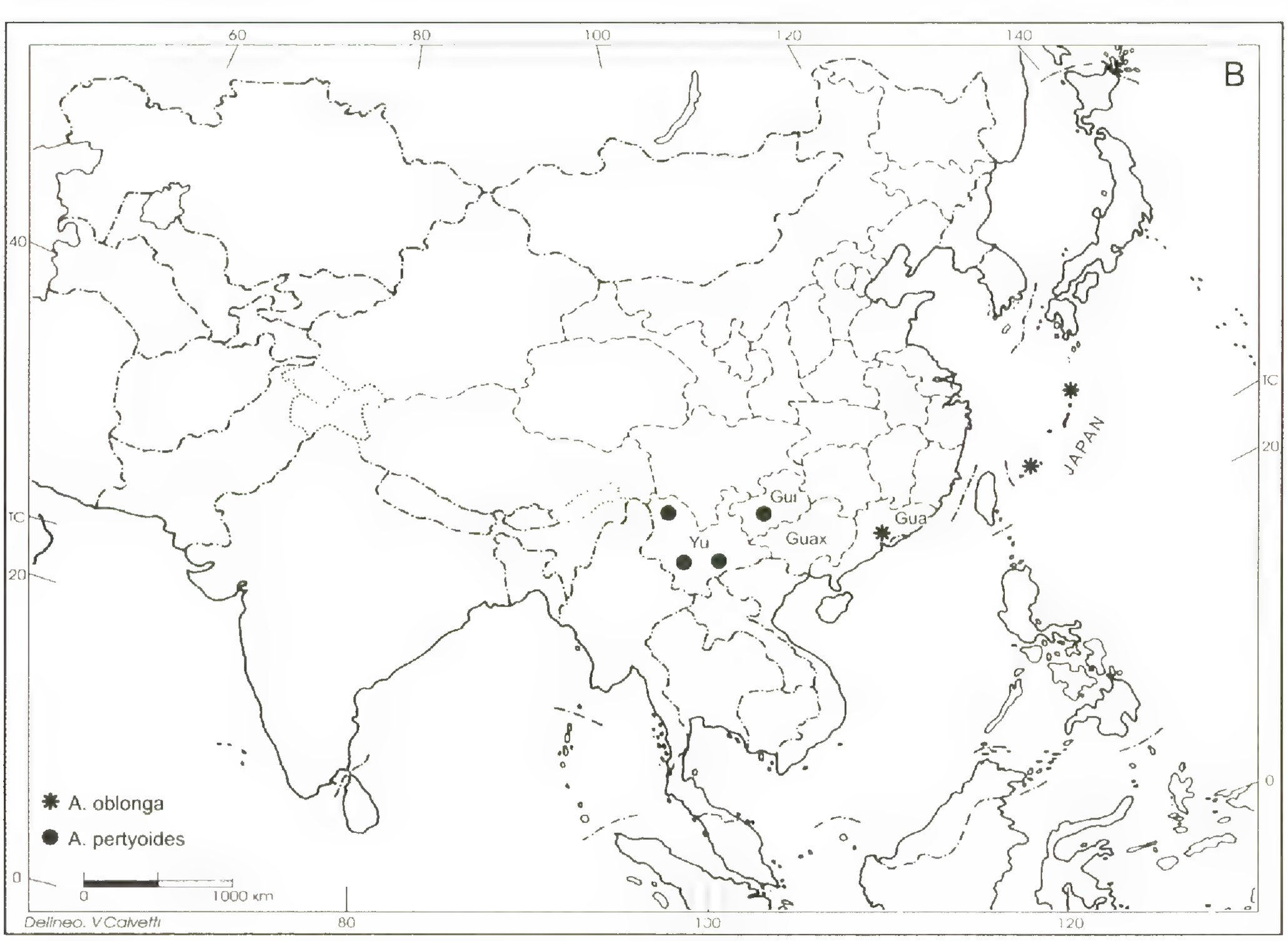
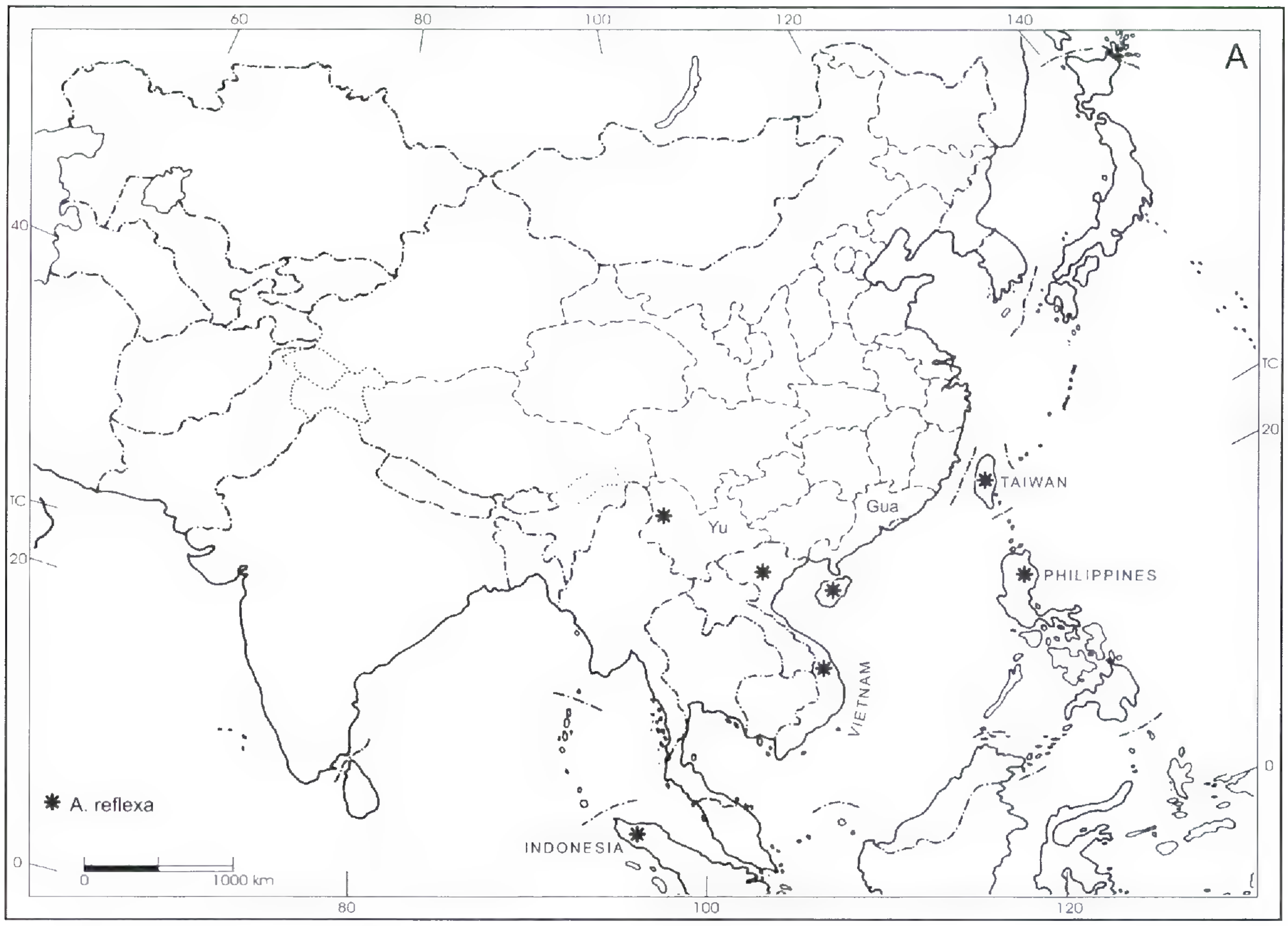


Figure 6. A. Distributions of *Ainsliaea kawakamii* (locality within Hunan unknown) and *A. macroclinidioides*. B. Distribution of *Ainsliaea oblonga* (locality within Jiangxi unknown) and *A. pertyoides*. An = Anhui; Fu = Fujian; Gui = Guizhou; Gua = Guangdong; Guax = Guangxi; Hun = Hunan; Yu = Yunnan; Zh = Zhejiang.



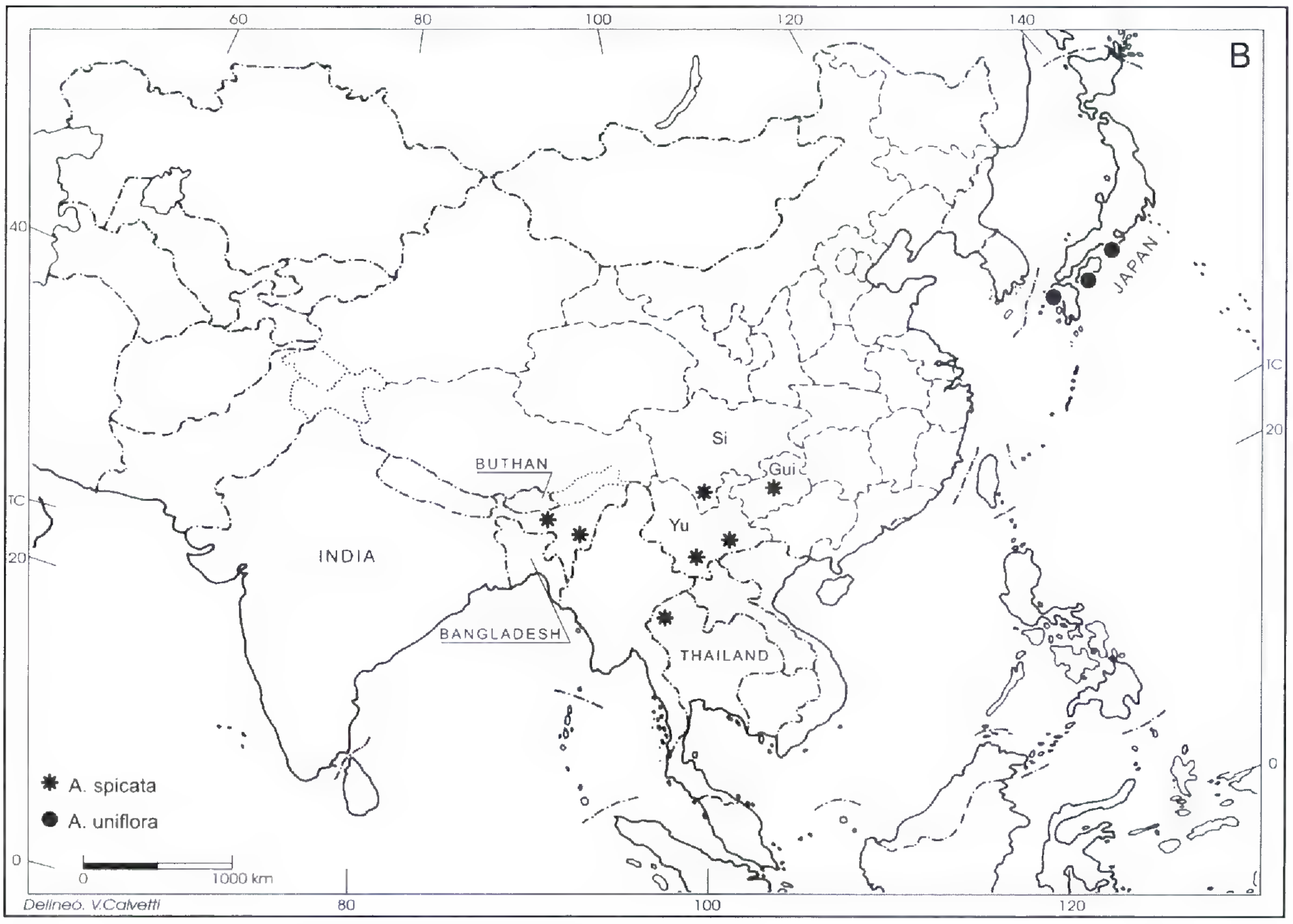


Figure 7. A. Distribution of *Ainsliaea reflexa*. —B. Distributions of *Ainsliaea spicata* (localities within Bangladesh and Bhutan unknown) and *A. uniflora*. Gui = Guizhou; Gua = Guangdong; Si = Sichuan; Yu = Yunnan.

apiculata, are found in both Japan and Korea (Fig. 3), and *A. acerifolia* reaches China (Liaoning).

There are several species, i.e., *Ainsliaea henryi*, *A. kawakamii* Hayata, *A. latifolia*, *A. reflexa*, that indicate a floristic relationship between Taiwan and China (Figs. 5–7). Others, such as *A. apiculata* and *A. macroclinidioides*, associate Taiwan with Japan (Figs. 3, 6). *Ainliaea fragrans* is present in all three: China, Taiwan, and Japan (Fig. 4).

Hu (1958) mentioned that many taxa in China showed an S-shaped distribution along the upper and middle Yangtze River, from Yunnan, Sichuan, and Hubei. Certain species of *Ainsliaea* are found in these provinces, e.g., *A. bonatii* (Fig. 3), *A. hypoleuca* (whose range extends to Vietnam, Fig. 5), *A. elegans*, *A. gracilis*, *A. grossedentata* (Fig. 4), *A. glabra* (ranging into Xizang on the west and Guizhou and Hunan in the east, Fig. 5), *A. henryi* (whose range extends to Fujian and Taiwan, Fig. 5), *A. pertyoides* (Fig. 6), and *A. spicata* (found from India and Bhutan and east to Thailand, Fig. 7).

According to the floristic regions of the world of Takhtajan (1986), *Ainsliaea* is present in three regions: most species occur in the Eastern Asiatic Region (corresponding to the Eastern Asiatic Kingdom of Wu & Wu, 1996), a few reach the Indochine Region (*A. brandisiana*, *A. chapaensis*, *A. elegans*, *A. hypoleuca*, *A. latifolia*, *A. pentaflora*, *A. reflexa*, *A. spanocephala*, *A. spicata*), and only two extend into the Malasian Region (*A. latifolia* and *A. reflexa*). The closer association between the Eastern Asiatic Region (principally southern Yunnan), Indochine Region (Vietnam, Thailand, and Hainan Island), and Malasian Region (Indonesia, Philippines) was previously pointed out by Zhu et al. (2003).

The distribution of the species of section *Ainsliaea* and section *Aggregatae* agrees in general with the two subkingdoms proposed by Wu and Wu (1996) within the Malasian Region, i.e., Sino-Himalayan Forest subkingdom and Sino-Japanese Forest subkingdom, respectively.

Species of *Ainsliaea* are found in warm temperate climates, growing in evergreen or mixed forests. A large majority seem to prefer the wettest conditions, often growing along shaded stream banks and in swampy thickets (*A. apiculata*, *A. glumacea*, *A. spanocephala*, *A. uniflora*, *A. walkeri*). Other taxa (*A. hypoleuca*, *A. latifolia*, *A. macrocephala*, *A. parvifolia*, *A. spicata*), however, prefer drier conditions, being found in full sun, openings in the forest, forest margins, and on grassy slopes. Arano (1965) suggested that the high endemism in China is the result of the deterioration of climatic conditions, which affected the distribution of *Ainsliaea* species because they become relictual in warmer climates.

PHYLOGENETIC ANALYSIS

The phylogenetic analysis resulted in three most parsimonious trees (MPTs), each with 210 steps, and a fit = 142.6 (fit rescaled 29%), with the strict consensus cladogram shown in Figure 8.

These MPTs differ in one respect only. The *Ainsliaea chapaensis–A. glabra–A. yunnanensis* clade in one tree is sister to the *A. fulvipes* Jeffrey & W. W. Sm.–*A. elegans–A. fragrans–A. cavaleriei–A. spanocephala–A. angustata–A. pingbianensis* clade, whereas in the other trees the *A. chapaensis–A. glabra–A. yunnanensis* clade falls within a polytomy. In one of two trees, the polytomy contains the *A. fulvipes–A. elegans–A. fragrans–A. cavaleriei–A. spanocephala–A. angustata–A. pingbianensis* clade, and in the third tree, which is identical to the strict consensus cladogram, the polytomy also contains the *A. latifolia–A. bonatii–A. foliosa–A. hypoleuca–A. reflexa–A. macrocephala–A. qianiana* S. E. Freire clade.

There was extensive homoplasy that, combined with the low number of characters, resulted in a lack of support for most branches. A high jackknife value was found for the sister genera *Macroclinidium* and *Ainsliaea* (84), and *Ainsliaea* also had a relatively high jackknife value (61). Four clades (Fig. 8) were supported: (1) all species of *Ainsliaea* (except *A. uniflora*) clade (52); (2) *A. linearis–A. oblonga–A. macroclinidioides–A. gracilis–A. parvifolia–A. apteroides–A. apiculata–A. acerifolia–A. grossedentata–A. cordifolia–A. dissecta* (53); (3) *A. grossedentata–A. cordifolia–A. dissecta* (55); and (4) *A. cordifolia–A. dissecta* (60).

Synapomorphies (cf. Table 1) are mapped onto the phylogeny in Figure 9.

The strict consensus cladogram (Fig. 8) shows that *Ainsliaea uniflora* is the first taxon to split off, followed by *A. lancangensis*. Next appear two clades, the clade *A. kawakami–A. trinervis* Y. C. Tseng–*A. linearis–A. oblonga–A. macroclinidioides–A. gracilis–A. parvifolia–A. apteroides–A. apiculata–A. acerifolia–A. grossedentata A. cordifolia–A. dissecta* and the clade leading to the remaining species in *Ainsliaea*. Jackknife values were not high (the majority of branches are not supported), but this may be expected for cladistic analyses based solely on morphological characters. However, good support was found for *Ainsliaea* as a whole, and therefore, *Ainsliaea* is well defined as a monophyletic group, with three synapomorphies supporting its distinction from *Macroclinidium*. These synapomorphies include few florets (Table 1, char. 22 [2, 3]) and plumose pappus bristles all the same length and width (Table 1, char. 26 [1], char. 28 [1]). Of these, character 22 reverses in *A. pentaflora* and *A. spanocephala* (Table 1, char. 22 [0]).

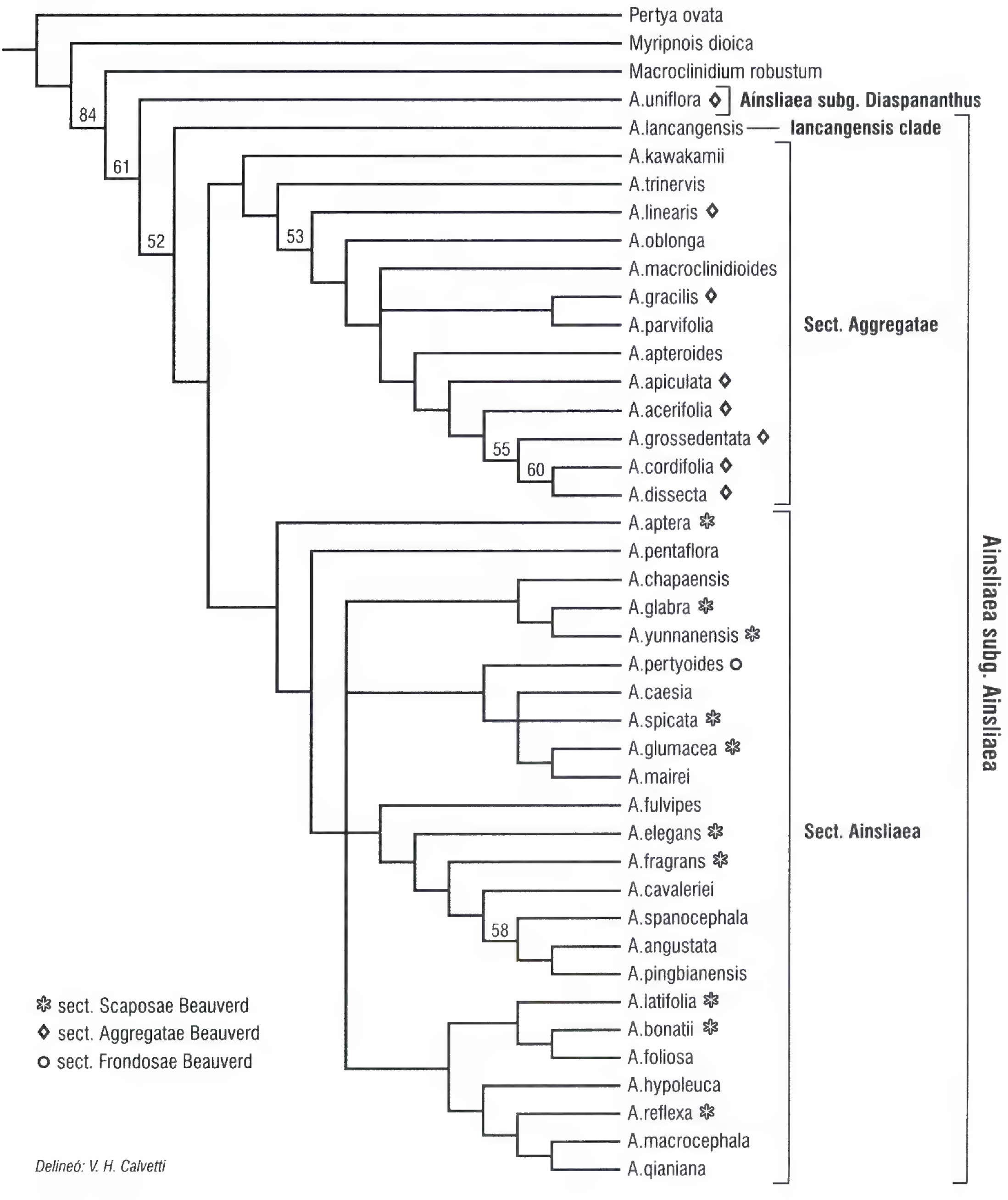


Figure 8. Strict consensus cladogram from three most parsimonious cladograms. Numbers above branches denote jackknife frequencies; only significance values (above 50) appear. Vertical bars delimit *Ainsliaea* subgenera and sections followed in the present study.

Important relationships within *Ainsliaea*, confirmed by the results of the phylogenetic analysis, have been recognized in previous taxonomic studies. The close relationship of *A. uniflora* (sometimes placed in the monotypic genus *Diaspananthus* Miq.) and the remaining species of *Ainsliaea* is in agreement with Kitamura (1938), who considered that *A. uniflora* belonged to the genus based on the plumose pappus, but as an isolated subgenus within *Ainsliaea*. This relationship is also supported by the analysis presented here. Beauverd (1909) presented a taxonomic arrangement of *Ainsliaea* based on plant habit in which the species were arranged in three sections: section *Scaposae*, section *Aggregatae*, and section *Frondosae* (see Taxonomic History). The most striking difference between the cladogram found in the present

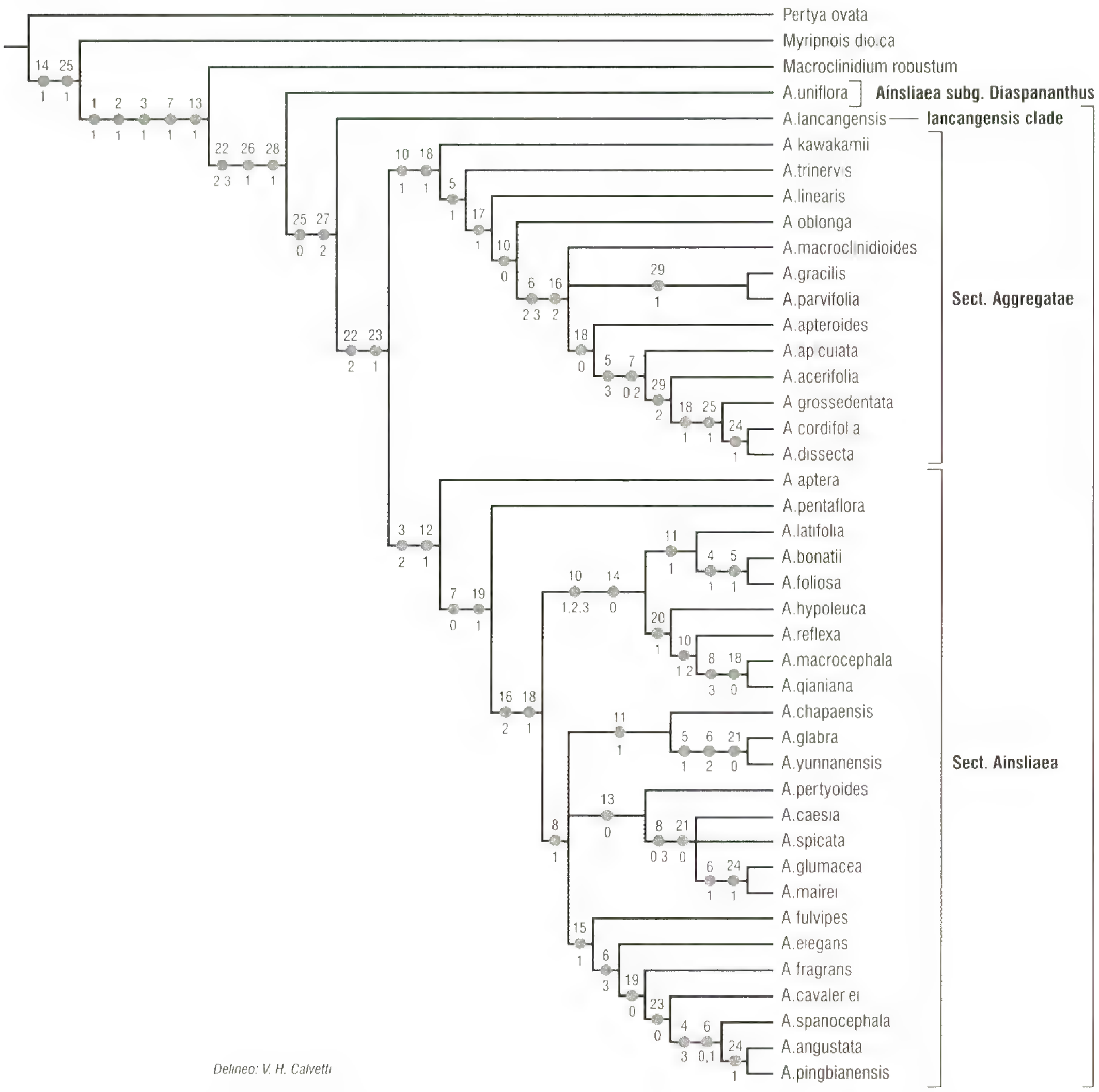


Figure 9. One of the most parsimonious cladograms found by Peewee (210 steps length, fit = 146.2, fit rescaled = 29%), showing all characters (numbers above gray dots; see Table 1) and states (numbers below gray dots; see Table 1) for clades.

analysis (Fig. 8) and Beauverd's sections is the position of *A. pertyoides*. This species is strikingly different from all other species in *Ainsliaea* (it has the greatest number, six, of apomorphies of the species, i.e., char. 1, state 0: shrub; char. 2, state 0: stem branched; char. 3, state 0: leaf alternate; char. 7, state 1: leaf acuminate; char. 16, state 0: capitula few on terminal leaf branchlets; char. 18, state 0: involucre 12–15 mm high). These features led Beauverd to place this species in a separate section (section *Frondosae*). However, Figure 8 shows that *A. pertyoides* is firmly positioned in the cladogram grouping with *A. caesia–A. spicata–A. glumacea–A. mairei*, within section *Ainsliaea*.

Based on the present analysis, a modified infrageneric classification for *Ainsliaea* is presented here and is indicated on the cladogram (Fig. 8): subgenus *Diaspananthus* (*A. uniflora*) and subgenus *Ainsliaea* with two sections, section *Ainsliaea* and section *Aggregatae*. Although they do not have strong support, these four subgeneric taxa are in accord with the remaining criteria outlined by Backlund and Bremer (1998) for general principles of classification, i.e., they are monophyletic and are recognizable based on morphological characters. Furthermore, these infrageneric clades, established as subgenera and sections by traditional systematists, have additional support from a historical perspective.

The isolated clade with *Ainsliaea lancangensis* is formally assigned to subgenus *Ainsliaea*, but it is difficult to assign it to any section based on morphological data, and it needs further investigation.

Taxonomic Treatment

Ainsliaea DC., Prodr. 7: 13. 1838. *Ainsliaea* subg. *Eu-Ainsliaea* Kitam., J. Jap. Bot. 14: 299. 1938. TYPE: *Ainsliaea pteropoda* DC. (= *Ainsliaea latifolia* (D. Don) Sch. Bip.), lectotype, designated here.

Perennial herbs, more rarely small shrubs, scapose, producing subterranean rhizomes, glabrous or densely pubescent with long crisped, straight, or T-shaped trichomes; stems erect, slender, terete, simple (occasionally branched and leafy above). Leaves simple, alternate, or, more commonly, in basal rosettes or clustered at the median part of the stem; sessile or distinctly petiolate with petioles alate or wingless; leaf blades mostly simple, entire or lobed; linear, ovate, elliptic, obovate, deltoid, or subrounded; chartaceous, subcoriaceous, or somewhat fleshy and subcarnose; base attenuate, cuneate, rounded to truncate, or subcordate to conspicuously cordate; margins denticulate-mucronate to dentate-mucronate; palmati-pinnate, 3- to 5-nerved or palmativeined, 7- to 9-nerved. Capitula homogamous, arranged in thyrsoid or stachyoid inflorescences, commonly nodding in anthesis; involucre often pluriseriate, cylindrical to narrowly obdeltate; peduncles commonly bracteolate, with upper bracts transitioning to involucre; phyllaries papyraceous or coriaceous, glabrous or pilose, usually ovate to ovate-lanceolate, acute or acuminate at the apex. Florets 1 to 5, hermaphrodite, chasmogamous or cleistogamous; corollas ligulate, larger than pappus bristles; lip irregularly deeply lobed, with lobes linear-oblong, reflexed and usually coiled; tube usually as long as the lip or slightly shorter (in chasmogamous florets) or pseudoligulate, tubular, shorter than pappus bristles, 4- or 5-lobed, with one deeper slit but without an expanded lip (in cleistogamous florets); glabrous, apically papillose; 5-nerved (marginal veins fused), without central veins; anthers 5, with apical appendages rounded to truncate or apiculate (more rarely emarginate); sagittate, with long tails smooth to slightly pilose or pilose; style branches bilobate to shortly branched, obtuse or rounded to truncate, dorsally shortly papillose. Achenes oblong to oblong-elliptic or oblong-obovate, usually those of cleistogamous florets larger than the chasmogamous florets; glabrous or pilose; (9)10-nerved, costate more prominent in glabrous or sparsely pilose achenes; pappus bristles usually uniseriate, more rarely biseriate; margins plumose.

Ainsliaea was circumscribed by A. P. de Candolle (Prodr. 7: 13. 1838) as containing two species (*A. pteropoda* and *A. aptera*), no species name being designated by de Candolle as the type. Conversely, no type species was mentioned by Kitamura (1938) for his subgenus *Eu-Ainsliaea*. According to the protologue, de Candolle's purpose was to transfer the species of D. Don (*Liatris latifolia*) to a new genus. Therefore, even if both species are in accordance with the protologue, *Ainsliaea pteropoda* DC. (= *Ainsliaea latifolia* (D. Don) Sch. Bip.) is here designated as lectotype of *Ainsliaea* DC.

KEY TO THE SUBGENERA, SECTIONS, AND "*LANCANGENSIS* CLADE" OF *AINSLIAEA* IN ASIA

1a. Pappus biseriate . . . . . I. subg. *Diaspananthus*
1b. Pappus uniseriate . . . . . II. subg. *Ainsliaea*
2a. Capitula 1-flowered . . . A. "*Lancangensis* clade"
2b. Capitula 2- to 5-flowered.
3a. Leaves in basal rosette (rarely alternate); capitula with peduncles, when present, ebracteate . . . . . B. sect. *Ainsliaea*
3b. Leaves usually clustered at the median part of the stem; capitula with peduncles, when present, usually bracteate . . . . . C. sect. *Aggregatae*

I. Ainsliaea subg. **Diaspananthus** (Miq.) Kitam., J. Jap. Bot. 14(5): 298. 1938. *Diaspananthus* Miq., Ann. Mus. Bot. Lugd. Bat. 2: 186. 1866, as "*Diaspanthus*." TYPE: *Diaspananthus uniflorus* (Miq.) Kitam. (= *Ainsliaea uniflora* Sch. Bip.).

Perennial herbs: stems erect, unbranched. Leaves in basal rosette. Capitula shortly pedunculate, arranged in thyrsoid inflorescences, nodding in anthesis; involucre cylindrical. Florets 1, hermaphrodite, chasmogamous; anthers with apical appendages apiculate and tails pilose; pappus bristles biseriate.

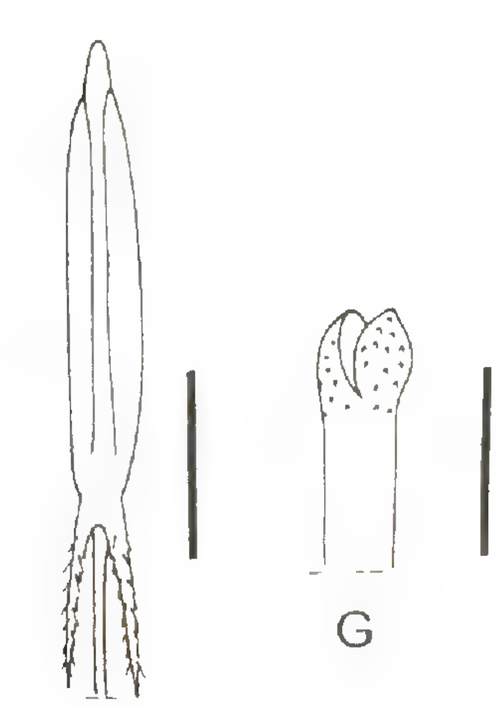
One species from Japan.

Discussion. Miquel (1866: 186) cited under his genus *Diaspananthus* one species name only, *D. palmatus* Miq., with *Ainsliaea uniflora* Sch. Bip. mentioned in parentheses immediately after the name, i.e., "*Diaspananthus palmatus* (*Ainsliaea uniflora* Sch. Bip., Pollichia 18: 187)." *Diaspananthus palmatus* is illegitimate, because it was a superfluous name that, when published, included the type *A. uniflora*, whose epithet ought to have been adopted.

Because *Diaspananthus* has corollas ligulate and plumose pappus, it is placed here as a subgenus within *Ainsliaea* as was proposed by Kitamura (1938: 298).

1. Ainsliaea uniflora Sch. Bip., in Jahresber. Pollichia 18: 187. 1861. *Diaspananthus uniflorus* (Sch. Bip.) Kitam., Mem. Coll. Sci., Kyoto Imp. Univ., ser. B, XV no. 3 (Comp. Jap. II) 305. 1940. TYPE: Japan. s.d., *H. Burger & H. Zollinger 283* (type, K!). Figure 10.

Plants perennial, herbaceous, 0.50–0.70 m tall; stems erect, unbranched below the inflorescence, naked, and sparsely strigose. Leaves basally clus-



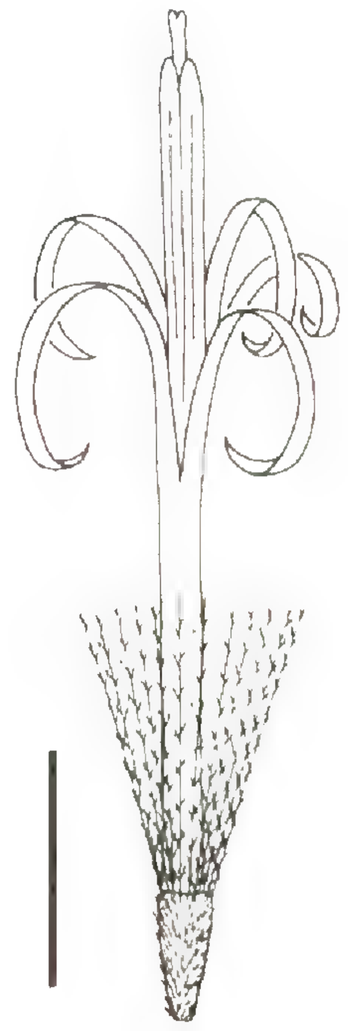


Figure 10. *Ainsliaea uniflora* Sch. Bip. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Phyllaries. —E. Chasmogamous floret. —F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 0.1 mm; C, E = 5 mm; D = 3 mm; F = 2 mm; G = 1 mm. Drawn from A, C–G, *Mimoro et al. 4197* (MO); B, (US 1132181).

tered, rosulate, long- petiolate; petioles wingless, flat, longer than the blades, 14–24 cm; leaf blades chartaceous, deeply 5- to 7-lobed, 8–12 × 9–13 cm, deeply cordate at the base, lobes irregularly sinuate-dentate and mucronate-toothed (apical lobe widest); green and subglabrous above, paler below and scarsely strigose with long straight trichomes; 5- to 7-palmativeined. Capitula pedunculate, arranged in thyrsoid inflorescence; peduncles 2–9 mm; involucre 5-seriate, cylindrical, ca. 11 × 2 mm; phyllaries papyraceous, purplish, glabrous; outer phyllaries ovate, subobtuse, 1–2 × 0.5–1 mm; inner phyllaries oblong-elliptic, subobtuse, 3-nerved, 9–10 × 1.5 mm. Florets 1, hermaphrodite, chasmogamous; corolla ligulate, ca. 20 mm, lobes ca. 7.5 mm (deeper slit ca. 10 mm), tube ca. 10 mm; anthers ca. 7 mm, apical appendages apiculate, ca. 0.7 mm, sagittate; tails ca. 2 mm, pilose; style branches short, obtuse to acute, ca. 0.5 mm. Achenes obovate, ca. 3 mm, 10-nerved, densely pilose; pappus bristles 65 to 70, ca. 6 mm.

Distribution and phenology. Japan (Honshu, Kyushu, Shikoku), collected in bodies of water, at elevations between 50 and 750 m (Fig. 7B); flowering in October.

Relationships. *Ainsliaea uniflora* may be distinguished from all other *Ainsliaea* species by its basal rosette of leaves that are deeply lobed, its capitulum of one floret, a biseriate pappus, and pilose anther tails.

Vernacular name. Kusa-yatsude (Iinuma, 1862).

Representative specimens examined. JAPAN. Honshu. **Mie:** Ikegawa, Arima-chô, Kumano-shi, *K. Seto 30054* (CM); Ise, Yamada, 10 Oct. 1911, s. coll. (US 1132181); Kii, Iwotobi, Owase-machi, Kita-muro-gun, *M. Furuse in 1956* (S, UC); Kii, Kitayama—river, Ooidani, Isato-machi, Kumano-shi, *M. Furuse in 1961* (S). **Nara:** Nojiri, Totsukawa-mura, Yoshino-gun, side of the R. 168, *K. Mimoro et al. 1197* (MO). **Wakayama:** Kobiro-tõge, Kogomori, Hongu-cho, Higasimuro-gun, along the R. 311, *K. Mimoro et al. 1117* (MO, NY). Kyushu. **Nagasaki:** Nagasaki, Naga-yama, *C. Maximowicz in 1863* (M, S, W). **Unknown:** Bungo, Sobosan, *T. Yamazaki 3103* (S). Shikoku. **Kochi:** Mt. Yokokura, Ochi-cho, Takaokagun, *K. Mimoro et al. 5151* (CM); Takaoka-gun, Ochi-machi, Yakokura-yama, *K. Shoma et al. in 1970* (MO). **Unknown:** Nanokawa, Tosa, 27 Sep. 1894, s. coll. (US 248115). **Unknown:** Japonia, *H. Burger s.n.* (S, W); Montagnes Syo?, *U. Faurie 11746* (MO).

II. Ainsliaea subg. **Ainsliaea** DC. *Ainsliaea* sect. *Scaposae* Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 376. 1909. TYPE: *Ainsliaea latifolia* (D. Don) Sch. Bip.

Ainsliaea sect. *Frondosae* Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 376. 1909. TYPE: *Ainsliaea pertyoides* Franch.

Perennial herbs (rarely small shrubs); stems erect, unbranched (rarely much branched and leafy above). Leaves in basal rosette or clustered near the median part of the stem (rarely alternate, loosely arranged). Capitula sessile or pedunculate, arranged in thyrsoid or stachyoid inflorescences, nodding in anthesis; involucre cylindrical to narrowly obdeltate. Florets 1 to 5, hermaphrodite, chasmogamous or cleistogamous; anthers with apical appendages rounded to truncate or apiculate (rarely emarginate) and tails smooth to slightly pilose or shortly pilose; pappus bristles uniseriate.

Forty-eight species from Afghanistan to Japan.

A. AINSLIAEA "*LANCANGENSIS* CLADE"

1. Ainsliaea lancangensis Y. Y. Qian, J. Trop. Subtrop. Bot. 8(2): 161. 2000. TYPE: China. Yunnan: Lancang, 2000 m, 26 Nov. 1993, *Y. Y. Qian 3185* (holotype, HITBC not seen; isotypes, IBSC, SMAO, not seen). Figure 11.

Plants perennial, herbaceous, 0.40–0.70 m tall; stems erect, unbranched below the inflorescence, glabrous. Leaves clustered at the median part of the stem, verticillate, long-petiolate; petioles wingless, flat, glabrous, (1.5)4–9 cm; leaf blades chartaceous, subrounded, 7- to 11-palmatifid, (2)4–11 × (1.5)5–12 cm, base cordate, slightly cuneate into the petiole, lobes triangular, acute to acuminate at the apex, margins mucronate-toothed; palmately nerved; slightly concolorous, upper surface glabrous, lower surface sparsely strigose with straight trichomes, and glandular capitate trichomes; palmativeined. Capitula pedunculate, arranged in thyrsoid inflorescence; peduncles 2–5 cm; involucre 8- or 9-seriate, cylindrical, ca. 14 × 2 mm; phyllaries papyraceous, sparsely strigose above; outer phyllaries ovate, obtuse, 1–1.3 × 0.7–0.8 mm; inner phyllaries linear-obovate, apiculate, conspicuously 3-nerved, 10–13 × 2.5–2.7 mm. Florets 1, hermaphrodite, chasmogamous; corolla ligulate, 1.3–1.7 cm, lobes ca. 9 mm (deeper slit ca. 10 mm), tube ca. 7 mm; anthers 7–7.5 mm, apical appendages apiculate, ca. 1 mm, sagittate; tails 1–1.3 mm, shortly pilose; style branches short, truncate, 0.4–0.5 mm. Achenes oblong, 6–7 mm, 10-nerved, densely pilose; pappus bristles ca. 37, 6.5–8.5 mm.

Type observation. Although neither holotype nor isotypes were available, it is a clear species from the description.

Distribution and phenology. At present, this is only known from the type collection from China (Yunnan, Lancang), from forest at 2000 m; flowering in November.

Figure 11. *Ainsliaea lancangensis* Y. Y. Qian. —A. Habit. —B, C. Foliar trichomes. —D. Capitulum. —E. Phyllaries. —F. Chasmogamous floret. —G. Stamen. —H. Style branches. Scale bars: A = 3 cm; B, C = 0.1 mm; D–F = 5 mm; G = 2 mm; H = 1 mm. Drawn from the paratype *Qian 3343* (SMAO).

Relationships. *Ainsliaea lancangensis* resembles *A. acerifolia* (principally from Japan) in its habit and leaves. Both species have leaves verticillate near the midpoint of the stem, palmately lobed leaf blades, apical anther appendages that are apiculate, and the capitula paniculate. *Ainsliaea lancangensis* is clearly distinguished by its capitula 1-flowered (vs. 3-flowered) and achenes densely pilose (vs. glabrous).

Representative specimen examined. CHINA. **Yunnan:** Lancang, *Y. Y. Qian 3343* (paratype, SMAO).

B. *AINSLIAEA* SECT. *AINSLIAEA* DC.

Ainsliaea sect. **Ainsliaea** DC. TYPE: *Ainsliaea latifolia* (D. Don) Sch. Bip.

Perennial herbs (rarely small shrubs); stems erect, unbranched (rarely much branched and leafy above). Leaves basally rosulate (rarely clustered above stem base or alternate and loosely arranged). Florets usually 3, sometimes 4 or 5, rarely 2; anthers with apical appendages commonly rounded, sometimes truncate or apiculate (rarely emarginate), and tails shortly pilose or sometimes smooth to slightly pilose.

Thirty-three species from Afghanistan, Bhutan, Southeast China (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Sichuan, Yunnan, and Xizang), India, Indonesia, Myanmar, Nepal, Pakistan, Philippines, Taiwan, Thailand, and Vietnam.

Key to the species of Ainsliaea *section* Ainsliaea *in Asia*

1a. Small shrubs; stems branched and sparsely leafy from the median part of the plant; floriferous branches leafy . . . . . 24. *A. pertyoides*

1b. Perennial herbs; stems simple or branched above the inflorescence; floriferous branches naked or with few reduced leaves.

2a. Leaf blades uniformly narrowed at the base.

3a. Leaves sericeous on both surfaces . . . . . 19. *A. mairei*

3b. Leaves glabrous or sparsely strigose below . . . . . 14. *A. glumacea*

2b. Leaf blades abruptly narrowed into the petiole.

4a. Leaves short-petiolate (petioles 4–10 mm long).

5a. Leaf blades lanceolate; achenes glabrous . . . . . 5. *A. caesia*

5b. Leaf blades obovate or obovate to pandurate; achenes pilose . . . . . 32. *A. spicata*

4b. Leaves long-petiolate (petioles more than 10 mm long).

6a. Petioles wholly winged (more than 1.5 mm wide at the upper point) or winged on the upper half.

7a. Leaf bases cordate.

8a. Phyllaries acuminate at the apex, dorsally glandular-pilose; capitula pedunculate . . . 30. *A. smithii*

8b. Phyllaries acute at the apex, apically strigose; capitula sessile . . . . . 3. *A. bonatii*

7b. Leaf bases rounded or attenuate.

9a. Involucre 12–18 mm long.

10a. Anther appendages truncate; phyllaries and leaves pilose . . . . . 18. *A. macrocephala*

10b. Anther appendages apiculate; phyllaries and leaves glabrous . . . . . 26. *A. qianiana*

9b. Involucre less than 10 mm long (occasionally equal to 10 mm).

11a. Leaves discolored, with upper surface deep green and lower surface white . . . 16. *A. hypoleuca*

11b. Leaves concolorous or only slightly discolorous.

12a. Leaves approximate above the base . . . . . 10. *A. foliosa*

12b. Leaves rosulate.

13a. Leaf margins straight; leaf bases rounded, petioles wholly winged . . . . . 17. *A. latifolia*

13b. Leaf margins wavy; leaf bases cuneate and commonly decurrent into the petiole (petioles winged on the upper half).

14a. Achenes glabrous or apically pilose . . . . . 15. *A. henryi*

14b. Achenes pilose . . . . . 28. *A. reflexa*

6b. Petioles wingless (leaf blades cuneate but petioles not winged in *A. glabra*, *A. nervosa*, *A. pentaflora*, *A. henryi*, *A. yunnanensis*).

15a. Capitula 5-flowered.

16a. Leaf blades ovate, leaf bases cuneate; foliar trichomes 1-seriate . . . . . 23. *A. pentaflora*

16b. Leaf blades oblong-elliptic; foliar trichomes 3-seriate.

17a. Leaf bases rounded . . . . . 31. *A. spanocephala*

17b. Leaf bases cordate . . . . . 4. *A. brandisiana*

15b. Capitula 2- or 3(4)-flowered.

18a. Leaf margins dentate.

19a. Blades acute to acuminate at the apex, slightly concolorous, sparsely strigose to subglabrous on both surfaces . . . . . 2. *A. aptera*

19b. Blades obtuse to rounded at the apex, discolorous, with upper surface lanuginose and lower surface white-tomentose . . . . . 8. *A. crassifolia*

18b. Leaf margins entire to denticulate.

20a. Leaf bases cordate.

21a. Capitula 2-flowered . . . . . 9. *A. elegans*
21b. Capitula 3(4)-flowered.
22a. Petioles densely strigose.
23a. Anther appendages emarginate; achenes pilose above . . . . . 11. *A. fragrans*
23b. Anther appendages apiculate; achenes pilose.
24a. Phyllaries apically pilose . . . . . 6. *A. cavaleriei*
24b. Phyllaries glabrous.
25a. Blades more or less concolorous. . . . . 27. *A. ramosa*
25b. Blades with lower surface whitish green, veins red . . . . . 29. *A. rubrinervis*
22b. Petioles lanuginose or glabrous.
26a. Blades ovate, 7–16 × 5–10 mm; achenes glabrous . . . . . 20. *A. nana*
26b. Blades subrotundate, 10–15 × 10–15 mm; achenes pilose . . . . . 22. *A. paucicapitata*
20b. Leaf bases rounded or cuneate.
27a. Petioles densely strigose.
28a. Leaf blades elliptic . . . . . 1. *A. angustata*
28b. Leaf blades oblong to elliptic.
29a. Blades 8–12 cm long; anther appendages apiculate . . . . . 25. *A. pingbianensis*
29b. Blades 3–7.5 cm long; anther appendages rounded . . . . 12. *A. fulvipes*
27b. Petioles lanuginose or glabrous.
30a. Blades elliptic, slightly fleshy, glabrous to subglabrous.
31a. Blades broadly elliptic, 3.5–6.5 mm wide; apex acute-acuminate . . . . . 13. *A. glabra*
31b. Blades linear-elliptic, 2–2.5 mm wide; apex acute . . . . 21. *A. nervosa*
30b. Blades ovate.
32a. Blades membranous, subglabrous.
33a. Achenes glabrous or apically pilose. . . . . 15. *A. henryi*
33b. Achenes pilose . . . . . 28. *A. reflexa*
32b. Blades coriaceous or subcoriaceous, strigose
34a. Involucre 13–15 mm long, foliar trichomes T-shaped . . . . . 33. *A. yunnanensis*
34b. Involucre 8–10 mm long, foliar trichomes straight . . . . . 7. *A. chapaensis*

1. **Ainsliaea angustata** C. C. Chang, Sinensia 5(1–2): 158. 1934. TYPE: China. Szechuan: Ma-Ping hsien, May 1930, *W. P. Fang 1641* (holotype, N not seen; isotype, PE not seen; PE photo LP!). Figure 12.

Plants perennial, herbaceous, 0.25–0.50 m tall; stems erect, apically branched, with few leaves much reduced, densely strigose. Leaves basally clustered, rosulate, long-petiolate; petioles wingless, 3–9 cm, densely strigose; leaf blades subcoriaceous, linear-elliptic to narrowly lanceolate, 4.5–9 × 1–1.9 cm, apex acute to obtuse-mucronate, base attenuate into the petiole, margins entire to shallowly denticulate-mucronate and strigose; strigose on both surfaces, with long straight trichomes, 1–1.5 mm; palmati-pinnate, 3-nerved; cauline leaves short-petiolate, linear-elliptic. Capitula short-pedunculate, arranged in thyrsoid inflorescence; involucre 5- to 6-seriate, cylindrical, 8–10 × 4 mm; phyllaries papyraceous, dorsally with glandular trichomes; outer phyllaries linear-ovate, acute at the apex, ca. 2.5 × 0.8 mm; inner phyllaries linear-elliptic, acute at the apex, 3- to 5-nerved, ca. 7.5 × 1.5 mm. Florets 3, hermaphrodite, chasmogamous; corollas light pink, ligulate, ca. 9 mm, lobes ca. 4 mm (deeper slit ca. 5 mm), tube ca. 4 mm; anthers purple, ca. 4.5 mm, apical appendages acuminate, 0.4–0.5 mm, sagittate; tails smooth to slightly pilose, 0.5–0.7 mm; style branches short, truncate, ca. 0.5 mm. Achenes oblong, 1.5–4 mm, 10-nerved, densely pilose; pappus bristles ca. 25 to 30, 6–6.5 mm.

Distribution and phenology. *Ainsliaea angustata* is known only from China (Sichuan), at ca. 1300 m; collected in flower in October.

Relationships. This species is characterized by its apiculate anther appendages, rosulate leaves with long strigose petioles, and narrow leaf blades. *Ainsliaea angustata* exhibits some likeness to *A. glumacea* in its habit and achenes. However, the latter species is amply distinguished by its rounded apical anther appendages and its petioles narrowly or broadly winged, and sparsely pilose. This species also resembles *A. fulvipes*, but *A. angustata* is distinct from *A. fulvipes* by its leaf blades linear to oblong (vs. oblong to elliptic), phyllaries glabrous (vs. pilose above), and apical anther appendages acuminate (vs. rounded).

Representative specimens examined. CHINA. **Sichuan:** Szechuan, Tien-chuan-hsien, *K. L. Chu 2273* (PE, W); Ma-

Figure 12. *Ainsliaea angustata* C. C. Chang. —A. Habit. —B. Foliar trichome. —C. Phyllaries. —D. Capitulum. —E. Chasmogamous floret. —F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 0.2 mm; C, E = 3 mm; D = 5 mm; F, G = 1 mm. Drawn from *Chu 2273* (PE).

Figure 13. *Ainsliaea aptera* DC. —A. Habit. –B, C. Foliar trichomes. —D. Capitulum. —E. Chasmogamous floret. —F. Style branches of chasmogamous floret. —G. Stamen of chasmogamous floret. —H. Cleistogamous floret. Scale bars: A = 3 cm; B = 0.1 mm; C = 0.2 mm; D, E = 5 mm; F, G = 2 mm; H =5 mm. Drawn from A, *Hooker f. & Thomson s.n.* (W); B, C, *Koelz 129* (NY); D–G, *Koelz 1789* (NY); H, *Kazmi in 1965* (M).

Ping hsien, *W. P. Fang 269* (paratype, N not seen); South Kansu, Pikow Mtns., *F. Kingdon Ward in 1910* (paratype, CGE not seen).

2. Ainsliaea aptera DC., Prodr. 7: 14. 1838. TYPE: Nepal. "India orientale," *Royle in 1833* (lectotype, designated here, K!). Figure 13.

Ainsliaea aptera f. *paniculata* Kuntze, Rev. Gen. Pl. 1: 304. 1891. Syn. nov. TYPE. India. Sikkim, 2400 m, Nov. 1875, *Herb. O. Kuntze 6956* (type, NY!).

Plants perennial, herbaceous, 0.30–0.75 m tall; stems erect, unbranched below the inflorescence, leafy, loosely strigose. Leaves basally clustered, 5–6(9) cm above stem base, long-petiolate; petioles wingless, flat, 5–13(20) mm, strigose; leaf blades chartaceous, ovate to deltate or deltoid, 5.5–15 × 4.5–12 cm, apex acute to acuminate-mucronate, base cordate, margins dentate; upper surface subglabrous, lower surface strigose, the surface paler below, with scarce glandular biseriate trichomes and long straight trichomes, 0.7–1 mm; palmati-pinnate, 5- to 7-nerved; cauline leaves alternate, short-petiolate to sessile, ovate to ovate-lanceolate. Capitula sessile or short-pedunculate (occasionally long-pedunculate), arranged in a stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, 8–15(22) × 2–4(6) mm; phyllaries coriaceous, glabrous or dorsally with glandular trichomes; outer phyllaries ovate, acute, 1.5–2 × ca. 1 mm; inner phyllaries linear-oblong, acute, 3-nerved, 11–14 × ca. 1.2 mm. Florets 3(4), hermaphrodite, chasmogamous and cleistogamous (at different or same seasons); corollas white with lavender center; *chasmogamous florets* with corolla ligulate, 11–13 mm, lobes ca. 7 mm (deeper slit ca. 8 mm), tube 4–5 mm; anthers purple, 3–4.5 mm, apical appendages rounded, ca. 0.8 mm, sagittate; tails ca. 1.5 mm, shortly pilose; style branches short, rounded, ca. 0.4 mm. Achenes oblong, (2)3–5 mm, 10-nerved, densely pilose; pappus bristles ca. 30 to 34(43), 5–8 mm; *cleistogamous florets* with corolla tubulose to pseudoligulate, 5–6 mm, 4- or 5-lobed, lobes ca. 0.2 mm (deeper slit ca. 0.3 mm); anthers much reduced, ca. 1 mm; style branches ca. 0.4 mm. Achenes 6–9 mm; pappus bristles 10–12 mm.

Distribution and phenology. A forest plant of Afghanistan, Bhutan, China (Xizang), India, Kashmir, Nepal, and Pakistan. It was collected from the floor of fir forest on damp organic soil, between 1200 and 3400 m (Fig. 3A); flowering from April to June and in November and December, fruiting from July to October and in March.

Discussion. De Candolle (1838) mentioned two syntypes in the protologue of *Ainsliaea aptera*, "*Wallich ann. 1821*" and "*Royle anno 1833.*" I found these two collections kept at GH and K, respectively. I propose the material *Royle in 1833*, which presents the most complete plant, as lectotype of *A. aptera*.

Ainsliaea aptera f. *paniculata* Kuntze was described on the basis of its capitula that are long-pedunculate and arranged in panicles. However, this is a variable feature throughout the range of *A. aptera*.

Relationships. *Ainsliaea aptera* most resembles *A. crassifolia*. Both taxa have leaves ovate to orbicular, with dentate margins and cordate at the base. However, the leaves in *A. aptera* are acute to acuminate at their apex and green-villose below. In contrast, *A. crassifolia* has leaves rounded at the apex and densely white-tomentose below.

Representative specimens examined. AFGHANISTAN. Herb. Kabulense, Konar, Dewagal Darrah Tal W des Chalas, Pass zum Darrah-e Mazar, *O. Anders 11129* (W). BHUTAN. Dochu La, pass betw. Thimbu & Wangdu Phodrang, *B. Bartholomew 1532* (US); 26 km W of Tongsa betw. Tongsa & Pele La, *B. Bartholomew & D. E. Bufford 3878* (NY). CHINA. **Xizang:** "Tibet," *Hügel 1241* (W). INDIA. **Assam:** Khasia, *J. D. Hooker & T. Thomson s.n.* (W). **Punjab:** Punjab, "prope Phagu ultra Simla, Sobha Ram," *J. R. Drummond 1846* (UC); Punjab, Simla, *J. R. Drummond 22635* (UC); Simla, *Hügel 719b* (W); Bushole, Rampur-Bishahr, Punjab, *W. Koelz 3168* (NY, US); Chaura, Tarand-Sarahan, Bashahr State, Punjab, *W. Koelz 7410* (NY). **West Punjab:** "Chandrakain" [Chandigarh], Kulu, Punjab, *W. Koelz 129* (NY, US); Kulu, Punjab, Naggar, *W. Koelz 1789* (NY); Kothi, Kulu, *Mohindar Nath 11* (NY). **Sikkim:** Sikkim, *J. D. Hooker & T. Thomson s.n.* (W). **Uttar Pradesh:** on way to Rifle range, Chakrata, Dehradun, *I. Kamini in 1961* (CANB); Chakrata, *M. B. Raizada in 1937* (S); Mussoorie, *H. Singh & S. Jalan in 1960* (AAU); Mussoorie, Jabberkhet, *R. R. Stewart 17166* (NY). **Unknown:** E Himalayas, *K. Biswas 3508* (NY); Ganges Valley, above Jálla, *J. F. Duthie 1739* (W); W Himalayas, *J. F. Duthie 19884* (W), E Himalaya, *Griffith 3330/1* (NY, W); Himalayas Bor., *J. D. Hooker & T. Thomson s.n.* (C, M, S, W); Dhakuri Pass, *W. Koelz in 1948, n. 20156?* (NY); "Gulwarg" [Godwar], *G. D. Sawson 15337* (MO, NY, UC, US); s. loc., *Sharp s.n.* (W); NW Himalayas, *Stolitzka in 1864* (W). KASHMIR. Lidder Valley, *J. F. Duthie 25653* (UC); *J. D. Hooker & T. Thomson s.n.* (W); Charehan, Patriata Murrel Hills, *R. R. Stewart 5203* (NY); Sonamarg, *R. R. Stewart 6836* (NY); Aru-Nafran, upper Lidder Valley, *R. R. Stewart 13506* (PENN); Sonamarg, Aug. 1978, *R. R. Stewart s.n.* (NY). NEPAL. Phulung Ghyang, Gossainkunde, *R. Maser 118* (US); "India orientale," *Wallich in 1821* (GH photo LP). PAKISTAN. "Hochwald oberhalb Shogran," Kaghantal, *E. J. Ecker 131* (W); Hazara, Kaghan, *S. M. A. Kazmi in 1965* (M); Changla Gali, Murrie Hills, *R. R. Stewart 10100B* (NY); Hazara, Changla Gali, *R. R. Stewart 27535* (W).

3. Ainsliaea bonatii Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 377. 1909. TYPE: China. Yunnan: "Yu nan sen," Tsong-chan, s.d., *F. Ducloux 3646* (type, NY!). Figures 14, 15.

Ainsliaea pteropoda var. *platyphylla* Franch., J. Bot. (Desvaux) 2: 69. 1888. Syn. nov. TYPE: China.

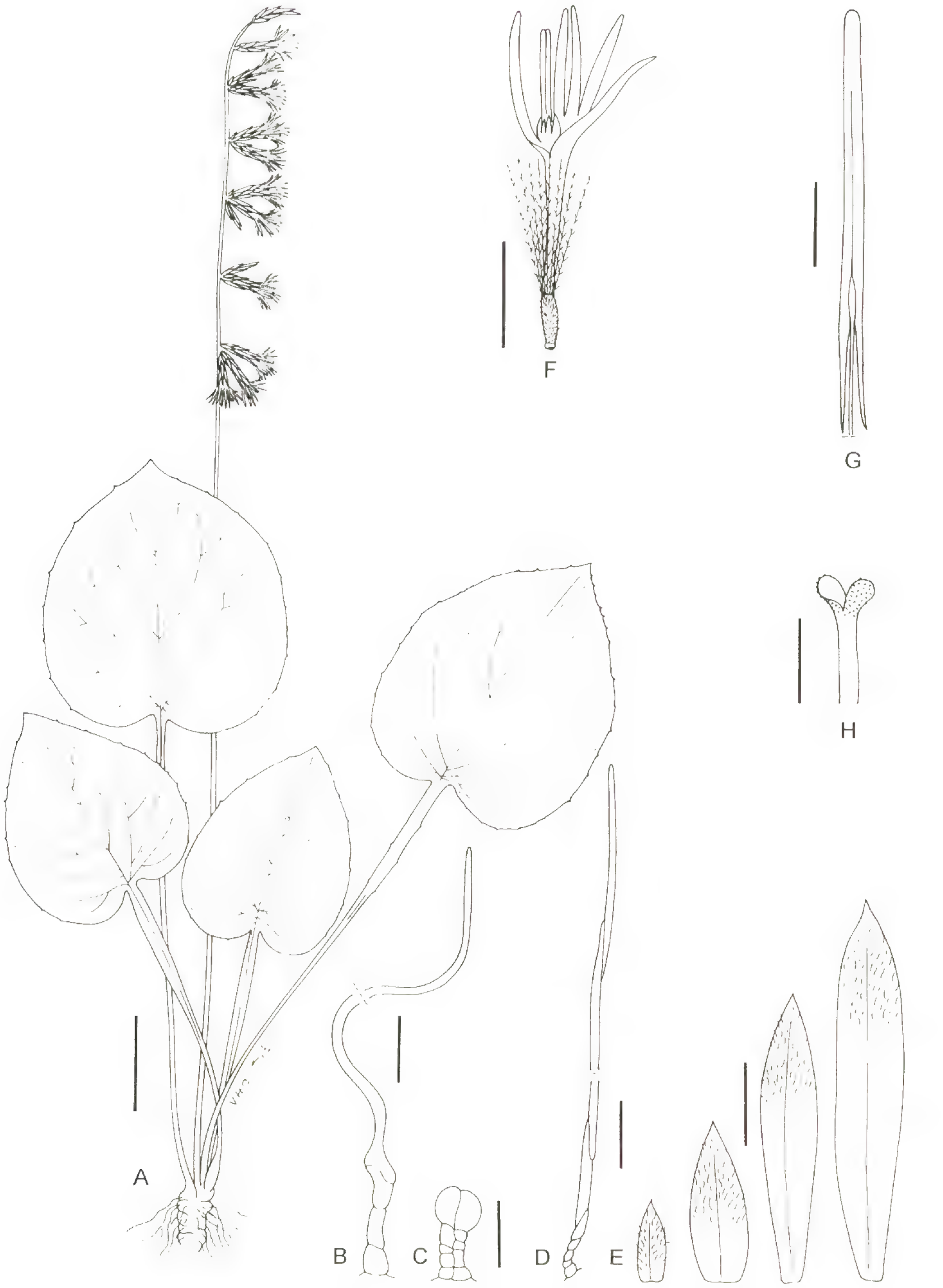


Figure 11. *Ainsliaea bonatii* Beauverd var. *bonatii*. —A. Habit. —B–D. Foliar trichomes. —E. Phyllaries. —F. Chasmogamous floret. —G. Stamen. —H. Style branches. Scale bars: A = 3 cm; B, C = 0.05 mm; D = 0.1 mm; E = 2 mm; F = 5 mm; G, H = 1 mm. Drawn from A, E–H, *Henry 9955* (MO); B–D, *Ducloux 3646* (NY).

Figure 15. *Ainsliaea bonatii* var. *multibracteata* (Mattf.) S. E. Freire. —A. Habit. —B. Cleistogamous floret. Scale bars: A = 3 cm; B = 2 mm. Drawn from *Smith 4745* (S).

Yunnan: Mo-Che-Tcho tze, 3 Oct. 1882, *J. M. Delavay 607* (lectotype, designated here, P!).

Ainsliaea bonatii var. *glabra* Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 377. 1909. TYPE: China. Yunnan: "Yu nan," Oct. 1906, *E. E. Maire 260* (holotype, P not seen).

Ainsliaea bonatii var. *arachnoidea* Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 378. 1909. TYPE: China. Yunnan: "Yu nan sen," 28 Oct. 1903, *F. Ducloux 253* (type, UC!).

Plants perennial, herbaceous, 0.20–0.70 m tall; stems erect, unbranched, loosely lanuginose. Leaves basally clustered, rosulate, longly petiolate with petioles broadly winged slightly narrowed to the base; leaf blades chartaceous, ovate to deltoid, cordate at the base, margins obscurely denticulate; upper surface sparsely strigose and lower surface white-strigose to lanate, with a combination of the following trichome types: short glandular biseriate trichomes, long crisped trichomes, and scattered long straight trichomes, ca. 2 mm; palmati-pinnate, 5- to 7-nerved. Capitula sessile, arranged in a stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, 10–13 × 3–4 mm; phyllaries papyraceous, apically strigose; outer phyllaries ovate, acute, 1.5–2 × 1–1.3 mm; inner phyllaries linear to oblong, acute, midvein dark, 10–13 × 0.6–1.2 mm. Florets 3(4), hermaphrodite, chasmogamous and cleistogamous (at different seasons or in single plant); corollas white; *chasmogamous florets* with corollas ligulate, (7)11–13.5 mm, lobes 0.5–5 mm (deeper slit (3)6–7.5 mm), tube (4)5–6 mm; anthers purple, 4.5–5 mm, apical appendages truncate, ca. 1 mm, sagittate; tails 1.5–1.8 mm, smooth to slightly pilose; style branches short, truncate, ca. 0.4 mm. Achenes oblong, 4–6 mm, densely pilose, 10-nerved; pappus bristles ca. 32, 5–8 mm; *cleistogamous florets* with corollas tubulose to pseudoligulate, 3–4 mm, 4- or 5-lobed, lobes 0.2 mm (deeper slit 0.3 mm); anthers ca. 1 mm; style banches ca. 0.7 mm. Achenes ca. 6 mm; pappus bristles ca. 32, 6–7 mm.

Distribution. China (Sichuan and Yunnan), at elevations between 2400 and 3500 m (Fig. 3B).

Discussion. Franchet (1888: 69) mentioned two syntypes in the protologue of *Ainsliaea pteropoda* var. *platyphylla*, "Mo-Che-Tcho tze, 3 Oct. 1882, *J. M. Delavay 607*" and "Mo-che-Tchin, supra Tapin-tze, 30 Nov. 1886, *J. M. Delavay 1029*." I selected as lectotype *J. M. Delavay 607* kept at P, since I was unable to see the syntype *J. M. Delavay 1029*.

Ainsliaea pteropoda var. *platyphylla* is placed into synonymy of *A. bonatii* Beauverd because there is no diagnostic character that differentiates the former from the latter.

Relationships. *Ainsliaea bonatii* most resembles *A. latifolia*. Both species have leaves broadly petiolate and lanose below (with long crisped trichomes). However, they may be differentiated by leaf bases, i.e., the leaves are rounded at the base in *A. latifolia*, whereas *A. bonatii* has cordate leaves.

Key to the varieties of Ainsliaea bonatii *in China*

1a. Leaf blade broadly ovate to orbicular . . . 3a. var. *bonatii*
1b. Leaf blade narrowly ovate . . . . 3b. var. *multibracteata*

3a. Ainsliaea bonatii Beauverd var. **bonatii**. Figure 14.

Stems with few leaves much reduced. Leaf blades broadly ovate to orbicular, 6–11.5 × 5–11.5 cm, apex acute to subobtuse; petioles 5–19 cm × 4–18 mm (at the upper point). Flowers usually chasmogamous, occasionally florets cleistogamous at the apex of the stem.

Distribution and phenology. Wet places on rocks under mixed conifer forest, between 2400 and 3000 m, from China (Sichuan and Yunnan); collected in flower in October and November.

Representative specimens examined. CHINA. **Sichuan:** 911 Forestry Center, 26 Aug. 1983, *s. coll. 46* (HITBC); Muli Co., Laluo, 1 Oct. 1983, s. coll. (HITBC 076240). **Yunnan:** "Luqiann" [Luquan] Co., Mt. Mumunshan, *Y. P. Chang 0694* (HITBC); Mo-che-Tchin, supra Tapin-tze, *J. M. Delavay 1029* (paratype of *Ainsliaea pteropoda* var. *platyphylla*, P not seen); Mi lê, *A. Henry 9955* (MO, NY); "Yu-nan-sen" [Hsiang-Yun], *E. E. Maire 920* (C, UC), *E. E. Maire 2349* (UC); Yunnan, W of Talifu, Mekong watershed, rt. to Youngchang & Tengyueh, *J. F. Rock 6679* (US); S Yunnan, "Molangpo bei Yüendjiang," *H. v. Wissmann 425* (W). **Unknown:** W. Yunchiang, *E. A. Wilson 183* (W).

3b. Ainsliaea bonatii var. **multibracteata** (Mattf.) S. E. Freire, comb. et stat. nov. Basionym: *Ainsliaea multibracteata* Mattf., Notizbl. Bot. Gart. Berlin-Dahlem 11: 106. 1931. TYPE: China. Sichuan: Sze-chuan, Nanch'uan, 14 Oct. 1891, *C. Bock et A. v. Rosthorn 1220* (lectotype, designated here, O!; duplicate, W!). Figure 15.

Ainsliaea mattfeldiana Hand.-Mazz., Acta Horti Gothob. 12: 347. 1938. Syn. nov. TYPE: China. Sichuan: Sze-chuan, Drogochi, in silva mixta, 3400–3500 m, 26 Sep. 1922, *H. Smith 4745* (holotype, W!; isotype, S!).

Stems leafy with reduced alternate leaves petiolate to sessile. Leaf blades narrowly ovate, 8.5–11 × 5–6 cm, apex acute; petioles 5–13 cm × 6–8 mm (at the upper point); cauline leaves alternate, petiolate to sessile, ovate to ovate-lanceolate. Flowers usually cleistogamous.

Distribution and phenology. At present known only from type collections from China (Sichuan),

between 3400 and 3500 m; flowering in September and October.

Discussion. *Ainsliaea bonatii* var. *multibracteata* is given varietal status because it possesses narrowly ovate leaves; otherwise it is typical of the species. Because no herbarium was mentioned for type collection of *A. multibracteata*, I propose the specimen kept at O as lectotype, which presents the most complete plant.

Ainsliaea mattfeldiana was described on the basis of its ovate leaves and cleistogamous florets (vs. broadly ovate leaves and chasmogamous florets in *A. bonatii*). *Ainsliaea mattfeldiana* is proposed here as a new synonym of *A. multibracteata* Mattf. (= *A. bonatii* var. *multibracteata*) because there is no diagnostic character that differentiates the former from the latter. In addition, cleistogamous florets is a variable feature within the range of *A. bonatii*.

4. Ainsliaea brandisiana Kurz, J. Asiat. Soc. Bengal 41(2): 318. 1872, non *Ainsliaea brandisiana* Koyama, Acta Phytotax. Geobot. 32: 60. 1981. TYPE: Myanmar. Martaban, s.d., *S. Kurz 865* (holotype, G!). Figure 16.

Plants perennial, herbaceous, 0.45–0.90 m tall; stems erect, unbranched below the inflorescence, naked and strigose. Leaves basally clustered, rosulate, long-petiolate; petioles wingless, conspicuously strigose, 7–14 cm; leaf blades chartaceous, oblong to elliptic, 10–12 × 4.5–6 cm, obtuse to subobtuse at the apex, base cordate, margins obscurely denticulate; upper surface sparsely strigose, lower surface strigose, with 3-seriate straight trichomes; palmati-pinnate, 3-nerved. Capitula long-pedunculate, arranged in thyrsoid inflorescence; involucre 5-seriate, campanulate, ca. 10 × 5 mm; phyllaries papyraceous, dorsally with glandular trichomes; outer phyllaries ovate, acuminate, ca. 3 × 0.4 mm; inner phyllaries linear to lanceolate, acute, 3- to 5-nerved, ca. 10 × 0.5 mm, acute. Florets 5, hermaphrodite, chasmogamous; corollas (missing, described from the original diagnosis) white, ligulate, ca. 15 mm. Achenes oblong-obovate, 4–5 mm, 10-nerved, densely pilose; pappus bristles ca. 34, ca. 6 mm.

Distribution and phenology. At present *Ainsliaea brandisiana* is only known from the type collection from Myanmar (Martaban) (Fig. 3B); flowering collection without month.

Relationships. *Ainsliaea brandisiana* is most similar to *A. spanocephala*. Both species have capitulum of five florets, leaf blades oblong to elliptic and foliar trichomes 3-seriate. However, *A. brandisiana* differs from this species by its leaf bases cordate (vs. rounded).

5. Ainsliaea caesia Hand.-Mazz., Beih. Bot. Centralbl. 56: 469. 1937. TYPE: China. Guangdong: Kwangtung, "in montibus Lungtou-schan 60 km ad orientem urbis Siudsao" [Schautschou], 900 m, 8 Oct. 1917, *R. Mell 874* (holotype, WU!). Figure 17.

Plants perennial, herbaceous, ca. 0.80 m tall; stems erect, unbranched, naked, with few leaves much reduced, strigose. Leaves basally clustered, rosulate, short-petiolate; petioles wingless, ca. 1 cm; leaf blades chartaceous, lanceolate, 5–5.5 × 12–13 cm, apex acute, rounded at the base, margins entire to obscurely denticulate; strigose on both surfaces and more dense along the margins with long straight trichomes; palmati-pinnate, 3-nerved. Capitula sessile, arranged in a stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, ca. 10 × 1.5 mm; phyllaries papyraceous, glabrous; outer phyllaries ovate, acute, ca. 2 × 1 mm; inner phyllaries linear to lanceolate, acute, 3-nerved, 10–13 × ca. 1.5 mm. Florets 3, hermaphrodite, cleistogamous; corollas tubulose to pseudoligulate, ca. 4 mm, lobes ca. 0.3 mm (deeper slit 0.5 mm); anthers ca. 2.5 mm, apical appendages truncate, ca. 0.4 mm, sagittate; tails ca. 1 mm, shortly pilose; style branches short, truncate, ca. 0.5 mm. Achenes oblong, ca. 1.5 mm, 10-nerved, glabrous; pappus bristles uniseriate, ca. 30, 7–8 mm.

Distribution and phenology. At present *Ainsliaea caesia* is only known from the type collection from China (Guangdong). It was collected on mountain slopes along river margins, on granitic soils at 900 m; flowering in October.

Relationships. *Ainsliaea caesia* resembles *A. glumacea* and *A. mairei* in its basal rosettes of leaves with narrow blades. However, *A. caesia* differs principally by its lanceolate leaf blades (vs. obovate in *A. glumacea* and elliptic in *A. mairei*) and leaves short-petiolate (vs. sessile in *A. glumacea* and *A. mairei*).

6. Ainsliaea cavaleriei H. Lév., Fl. Kouy-Tchéou: 82. 1914. TYPE: China. Sud de Tin-fan, Nov. 1904, *J. Cavalerie 1907* (holotype, E!). Figure 18.

Ainsliaea cleistogama C. C. Chang, Sinensia 4: 225. 1934. Syn. nov. TYPE: China. Guangdong: Kwangtung, Tai Young Mt., 19 July 1921, 700 m, *F. A. McClure 459* (holotype, K not seen; isotype, NY!).

Plants perennial, herbaceous, 0.25–0.80 m tall; stems erect, unbranched below the inflorescence,

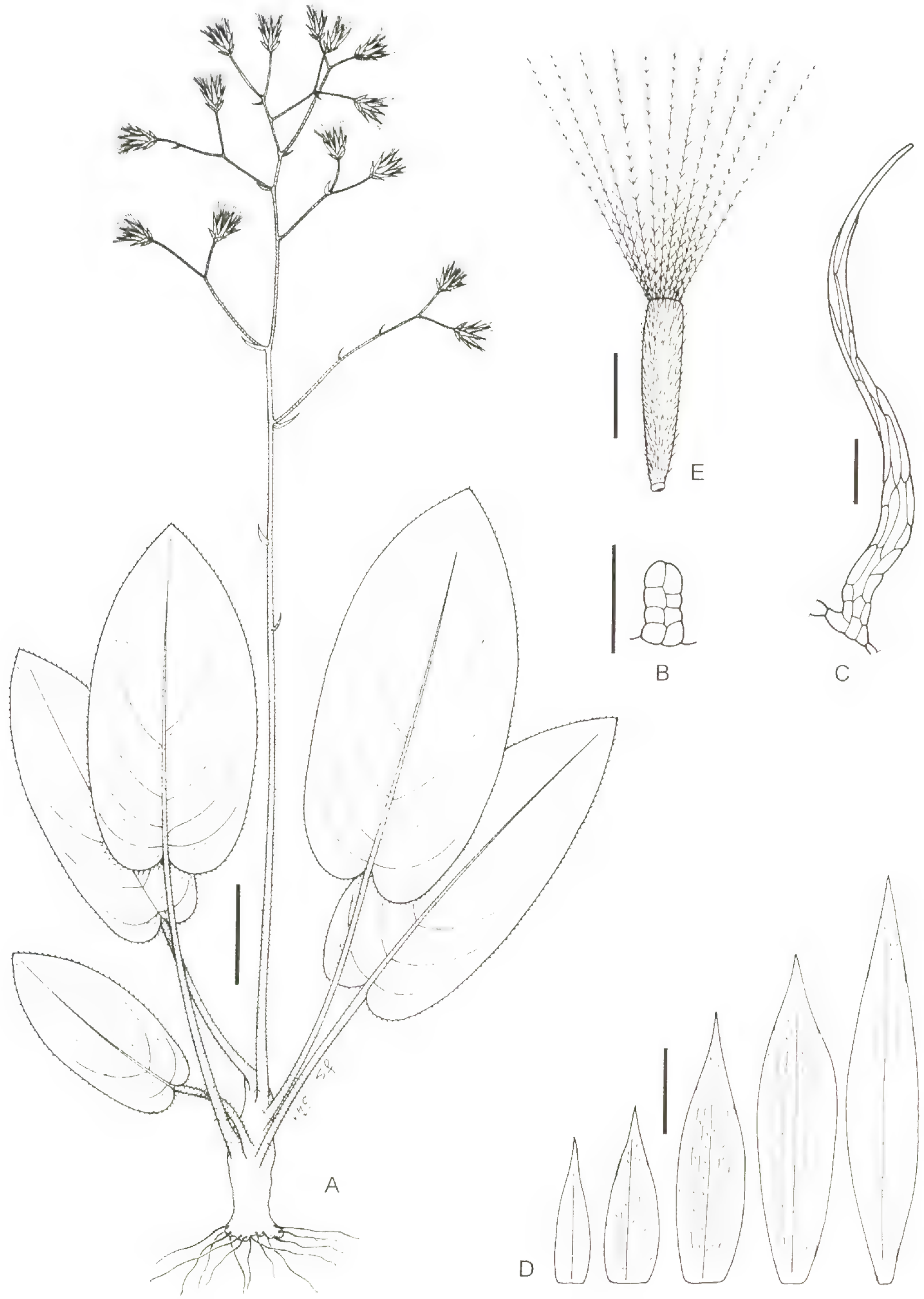


Figure 16. *Ainsliaea brandisiana* Kurz. —A. Habit. —B. Trichome of phyllaries. —C. Foliar trichome. —D. Phyllaries. —E. Achene with pappus. Scale bars: A = 3 cm; B = 0.05 mm; C = 0.1 mm; D, E = 2 mm. Drawn from the holotype *Kurz 865* (G).

Figure 17. *Ainsliaea caesia* Hand.-Mazz. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Phyllaries. —E. Cleistogamous floret. —F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 0.1 mm; C = 5 mm; D = 4 mm; E = 2 mm; F, G = 1 mm. Drawn from the holotype *Mell 874* (WU).

Figure 18. *Ainsliaea cavaleriei* H. Lév. —A. Habit. —B. Capitulum. —C. Phyllaries. —D. Foliar trichome. —E–H. Cleistogamous floret: E, corolla; F, stamen; G, style branches; H, achene with pappus. Scale bars: A = 3 cm; B = 5 mm; C = 2 mm; D = 0.1 mm; E = 1 mm; F, G = 0.5 mm; H = 2 mm. Drawn from *Steward & Cheo 1090* (S).

naked, with few leaves reduced, or scarcely leafy, strigose. Leaves basally clustered, rosulate, long-petiolate with petioles wingless, conspicuously strigose, (1.5)3–10 cm; leaf blades chartaceous, ovate to oblong, 4–13 × (0.5)3–7 cm, acute to subobtuse at the apex, base deeply cordate, margins obscurely denticulate; with upper surface sparsely strigose, and lower surface reddish-strigose, especially on the nerves (with long straight trichomes ca. 1.5 mm); palmati-pinnate, 3- to 5-nerved; cauline leaves (when present) similar to the radical, 17–30 × 9–16 mm, shortly petiolate and cuneate at the base. Capitula sessile or shortly pedunculate, arranged in thyrsoid inflorescence; involucre 5- or 6-seriate, cylindrical, 6–10 × 3–5 mm; phyllaries papyraceous, purple, strigose above; outer phyllaries ovate, acuminate at the apex, 2–2.5 × ca. 1 mm; inner phyllaries linear to elliptic, acute, 3- to 5-nerved, 6–10 × ca. 1 mm. Florets 3, hermaphrodite cleistogamous; corollas white, tubulose to pseudoligulate, 3–3.5 mm, 5-lobed (deeper slit 0.3–1.5 mm); anthers ca. 1.5 mm, apical appendages shortly apiculate, ca. 0.3 mm, sagittate; tails ca. 0.5 mm, shortly pilose; style branches short, rounded, ca. 0.4 mm. Achenes oblong-elliptic, 3–4 mm, 9-nerved, densely pilose; pappus bristles 28 to 34, ca. 6 mm.

Distribution and phenology. Southern China (Guangdong, Guangxi, Jiangxi) in moist slopes between 380 and 1050 m; collected in flower from May to July and from October to December, in flower bud in April and September.

Discussion. I propose to place *Ainsliaea cleistogama* into synonymy of *A. cavaleriei*, because there is no character that differentiates the former from the latter.

Relationships. *Ainsliaea cavaleriei* most resembles the species complex with *A. fragrans*, *A. ramosa*, and *A. rubrinervis*. This complex and *A. fragrans* share leaves ovate to oblong, deeply cordate at the base, and with villous petioles. However, *A. cavaleriei* can be differentiated by its apical anther appendages that are apiculate and its phyllaries strigose above.

Representative specimens examined. CHINA. **Guangdong:** Canton, *L. Deng 8023* (MO); Canton, *L. Deng 8878* (MO); Huaiji, *K. L. Shi 15077* (AAU); Sha Lo Shan, Wa Mei T'ong Village, Sin-fung district, *Y. W. Taam 113* (MICH, US); Conghua Co., near Sanchuan hydroelectric station, *X. W. Wang et G. C. Zhang 8241* (MO). **Guangxi:** Guangxi, *D. Chen 425* (MO); Guangxi, *S. Q. Chen 14753* (MO); Guangxi, *S. Q. Chen 17243* (MO); Guangxi, Ta Tseh Tsuen, Yung Hsien, *A. N. Steward & H. C. Cheo 1090* (NY, S, W); Guangxi, *F. Wei 230* (MO). **Jiangxi:** "Kiangsi," Yut Chi Fa, Oo Chi Shan near Lam Uk Tung Village, Lungnan district, *S. K. Lau 4554* (S, US). **Unknown:** *Q. Lu & Y. Wei 20097* (MO).

7. Ainsliaea chapaensis Merr., J. Arnold Arbor. 21: 387. 1940. TYPE: Vietnam. Tonkin: Chapa, 1500 m, Apr. 1938, *A. Pételot 2068* (holotype, GH not seen; isotypes, MO!, NY!, US!). Figure 19.

Plants perennial, herbaceous, 0.18–0.35 m tall; stems erect, shortly unbranched below the inflorescence, leafy, strigose. Leaves basally clustered, rosulate, long-petiolate, petioles wingless, 1.5–4.5 cm, strigose subglabrate at maturity; leaf blades subcoriaceous, ovate, 2.5–4.5 × 1.5–3 cm, apex acute to shortly acuminate, base broadly rounded, margins obscurely denticulate; subconcolorous, upper surface green-olivaceous, subglabrous, lower surface paler, moderately strigose, especially on veins, with long straight trichomes and short glandular trichomes; palmati-pinnate, 3-nerved; cauline leaves alternate, short-petiolate to sessile, ovate to lanceolate or elliptic, gradually reduced upward to the base of inflorescence. Capitula sessile, arranged in a stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, ca. 10 × 5 mm; phyllaries subcoriaceous, strigose; outer phyllaries ovate, acuminate at the apex, 1.5–2.5 × ca. 1 mm; inner phyllaries linear to lanceolate, acute, 3-nerved, 7.5–9 × 1.5–3 mm. Florets 3 to 4, hermaphrodite, chasmogamous; corolla ligulate, ca. 9 mm, lobes 2–3 mm long (deeper slit ca. 4 mm), tube ca. 5 mm; anthers 3.5–4 mm, apical appendage rounded, ca. 0.5 mm; sagittate, tails ca. 1.5 mm, shortly pilose; style branches short, rounded, ca. 0.5 mm. Achenes oblong to obovate, 2.5–3 mm, 10-nerved, densely pilose; pappus bristles ca. 28, 6–7 mm.

Distribution and phenology. At present is only known from the type collection from Vietnam, on very dry slopes in full sunlight, between 1500 and 1900 m (Fig. 3B); flowering in April.

Relationships. *Ainsliaea chapaensis* resembles *A. parvifolia* in its long-petiolate leaves, with the leaf blades ovate and capitula sessile. *Ainsliaea chapaensis* is distinguished by its leaf blades broadly rounded at the base (vs. cuneate) and its phyllaries strigose (vs. glabrous).

8. Ainsliaea crassifolia C. C. Chang, Sinensia 6(5): 549. 1935. TYPE: China. Yunnan: Yung-pe Mtns., 26°45′N, 10,000 ft., Sep. 1913, *G. Forrest 11070* (lectotype, designated here, E not seen; duplicates, E photo LP!, PE!). Figure 20.

Plants perennial, herbaceous, 0.60–0.75 m tall; stems erect, unbranched, leafy, densely white-lanuginose. Leaves basally clustered, rosulate, long-petiolate; petioles wingless, 4–11 mm, densely white-

Figure 19. *Ainsliaea chapaensis* Merr. —A. Habit. —B. C. Foliar trichomes. —D. Phyllaries. —E. Capitulum. —F. Chasmogamous floret. —G. Style branches. —H. Stamen. Scale bars: A = 3 cm; B = 0.1 mm; C = 0.05 mm; D, F = 3 mm; E = 5 mm; G, H = 1 mm. Drawn from the isotype *Pételot 2068* (MO).

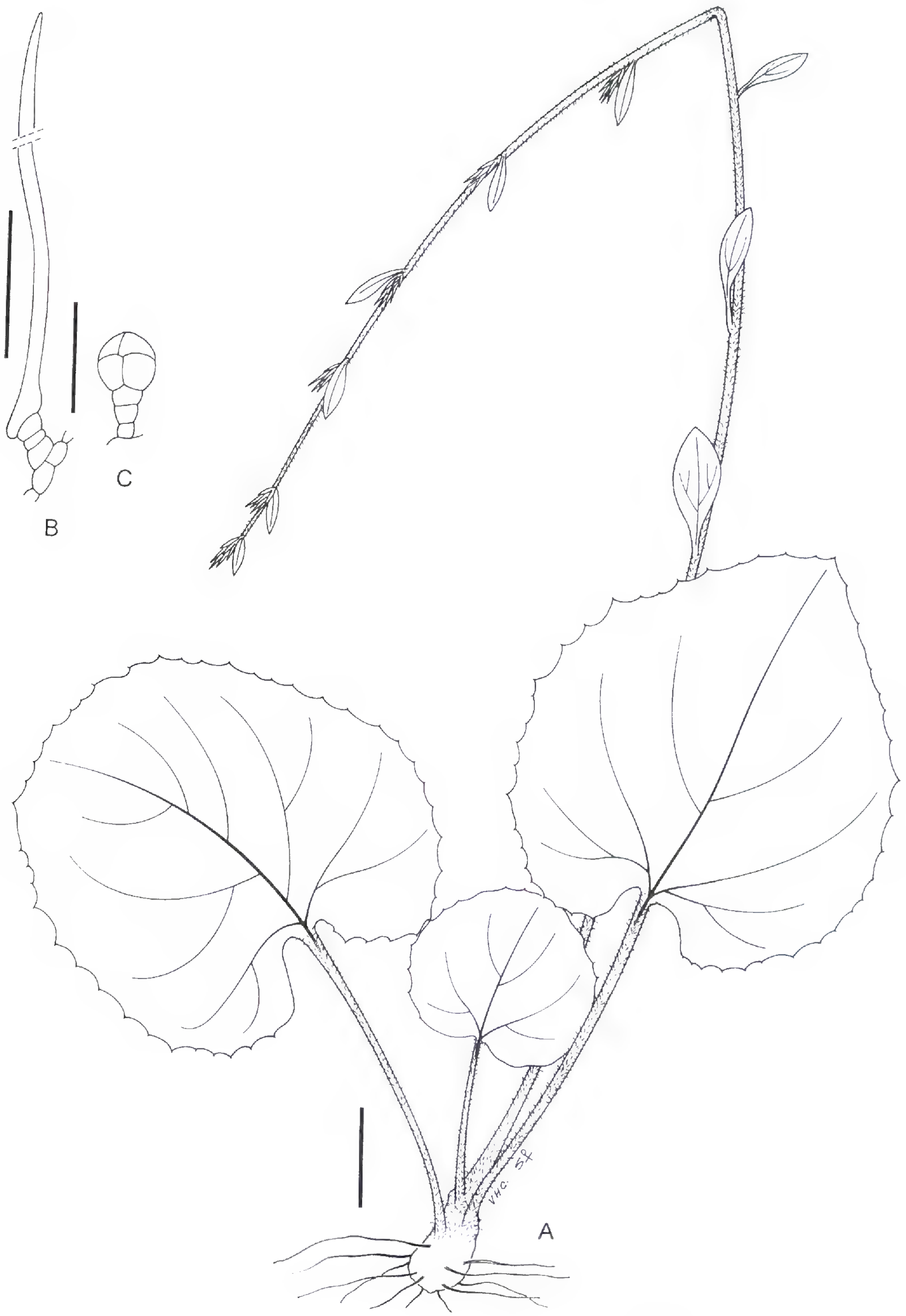


Figure 20. *Ainsliaea crassifolia* C. C. Chang. —A. Habit. —B, C. Foliar trichomes. Scale bars: A = 3 cm; B = 0.1 mm; C = 0.05 mm. Drawn from the lectotype *Forrest 11070* (PE).

lanuginose; leaf blades chartaceous, reniform, 9–13 × 6–12 cm, apex obtuse to rounded, base deeply cordate, margins dentate; strongly discolorous, with upper surface deep green, sparsely lanate, and lower surface densely white-lanate, with long crisped trichomes and capitate glandular trichomes; palmati-pinnate, 3- to 5-nerved. Cauline leaves alternate, ovate, ca. 5 × 3.5 cm, petiolate, petioles ca. 5 cm, gradually reduced upward to the base of the inflorescence. Capitula sessile, arranged in a stachyoid inflorescence; involucre papyraceous, glabrous.

Distribution and phenology. *Ainsliaea crassifolia* is only known from China (Yunnan and Sichuan), growing at 3000 m in open pasture on the margins of pine forest and in shady stony pasture beneath cliffs; only known from immature collections (September).

Discussion. Chang (1935) mentioned two syntypes in the protologue of *Ainsliaea crassifolia*, "Tong-shan in the Yangtze bend, Sep. 1913, *G. Forest 11319*" and "Yung-pe mountains, Sep. 1913, *G. Forrest 11070* " kept at E. I have seen a duplicate of the syntype *G. Forest 11070* at PE, which is in accordance with the protologue, so I selected the specimen *G. Forrest 11070* at E as lectotype.

Relationships. Although the floral characters of *Ainsliaea crassifolia* are unknown (also absent in the original diagnosis), it closely resembles the genus *Ainsliaea* in habit, leaves, and foliar trichomes, so the species is referred here, until further study.

Representative specimens examined. CHINA. **Sichuan:** Muli Co. at the bank of Donglanghe, *s. coll.* (HITBC 076241); **Yunnan:** Tong-shan in the Yangtze bend, *G. Forrest 11319* (paratype, E not seen).

9. Ainsliaea elegans Hemsl., Hooker's Icon. Pl. 28(2): t. 2747. 1902, non *Ainsliaea elegans* Hayata, Bot. Mag. (Tokyo) 20: 14. 1906. TYPE: China. Yunnan: Mengtze, 7000 ft., *A. Henry 9108* (lectotype, designated here, K!; duplicates, MO!, NY!). Figures 21, 22.

Pertya esquirolii H. Lév., Bull. Acad. Int. Géogr. Bot. 24(295–297): 251. 1914. TYPE: China. Guizhou: Kouy-Tcheou [Kweichow], Ta Pin et Than lo, 1150 m, Dec. 1912, *J. Esquirol 3633* (holotype, E!).

Ainsliaea elegans var. *tomentosa* Mattf., Notizbl. Bot. Gart. Berlin-Dahlem 11(102): 107. 1931. TYPE: China. Guizhou: Kouy-Tcheou [Kweichow], Ta Pin et Than lo, 1150 m, Dec. 1912, *J. Esquirol 3633* (holotype, E!).

Plants perennial, herbaceous, 0.30–0.70 m tall; stems erect, unbranched below the inflorescence, villose. Leaves basally clustered, rosulate, long-petiolate with petioles wingless, conspicuously villose, (6)16–30 cm long; leaf blades subcoriaceous, ovate to deltoid, (10)13.5–17.5 × 6.5–13 cm, acute to subobtuse at the apex, base deeply cordate, margins obscurely denticulate; palmati-pinnate, 3- to 5-nerved. Capitula sessile or shortly pedunculate, arranged in thyrsoid inflorescence; involucre 5- or 6-seriate, cylindrical, 12–13 × 4–5 mm; phyllaries subcoriaceous, dorsally pilose; outer phyllaries ovate, acute, ca. 4 × 1.5 mm; inner phyllaries linear to elliptic, shortly acuminate, midvein dark, ca. 12 × 2 mm. Florets 2 (very occasionally in the same plant capitula with 3 florets), hermaphrodite, chasmogamous; corollas white or pink, ligulate, ca. 16 mm, lobes ca. 7.5 mm (deeper slit ca. 9 mm), tube ca. 7 mm; anthers purple, ca. 6 mm, apical anther appendages rounded to slightly emarginate, 1.5 mm, sagittate; tails ca. 2 mm, shortly pilose; style branches short, truncate, ca. 0.4 mm. Achenes oblong, ca. 3 mm, 10-nerved, densely pilose; pappus bristles ca. 30, ca. 7.5 mm.

Distribution. Southern China (Guizhou, Yunnan) and Vietnam (Chapa), between 1150 and 2500 m (Fig. 4B).

Discussion. Two syntypes are mentioned in the protologue of *Ainsliaea elegans*, "*Henry 9108*" and "*Henry 9108A*." Both were collected at Mengtze, Yunnan (China), between 7000 and 8000 ft. I found the syntypes "*Henry 9108*" kept at K, MO, and NY, which are in complete accordance with the protologue. According to Stafleu and Cowan (1979), Hemsley's material can be found in K, so I choose as lectotype the specimen deposited at this herbarium.

Key to the varieties of Ainsliaea elegans *in China and Vietnam*

1a. Leaves densely white strigose-lanate on lower surface . . . . . 9a. var. *elegans*
1b. Leaves strigose on both surfaces . . . 9b. var. *strigosa*

9a. Ainsliaea elegans Hemsl. var. **elegans**. Figure 21.

Stem naked, with few leaves reduced. Leaves strongly discolored, with lower surface white strigose-lanate with two trichome types, long crisped trichomes and long straight trichomes, and the upper surface dark green, with long straight trichomes only.

Distribution and phenology. Southern China (Guizhou, Yunnan) in mixed forest between 1150 and 1900 m; collected in flower bud from October to December (flowering collections without date).

Relationships. *Ainsliaea elegans* var. *elegans* resembles *A. fragrans*. Both species have leaves deeply cordate at the base, villose petioles, and rounded

Figure 21. *Ainsliaea elegans* Hemsl. var. *elegans*. —A. Habit. —B, C. Foliar trichomes. —D. Phyllaries. —E. Capitulum. —F. Chasmogamous floret. —G. Stamen. —H. Style branches. Scale bars: A = 3 cm; B, C = 0.1 mm; D, E, F = 5 mm; G = 2 mm; H = 1 mm. Drawn from: A, C–H, *Henry 9108* (NY); B, *Wang 82572* (KUN).

anther appendages. *Ainsliaea elegans* var. *elegans* is distinguished by its ovate to deltoid leaves (vs. ovate to oblong), with blades densely white tomentose on the lower surface (vs. commonly purplish) and its capitulum of two florets (vs. three florets).

Representative specimens examined. CHINA. **Yunnan:** Mengtze, *Henry 9108A* (paratype of *Ainsliaea elegans*, not seen); Mar-li-po, Kwang-jin-in, *K. M. Feng 12964* (KUN); S Yunnan, Molangpo bei "Yuendjiang" [Yuanjiang], *H. t. Wissmann 456* (W); "Y. Ping-bien Hsien" [Pingbian], Ji-mu-te, ta-nei-shan, *C. W. Wang 82572* (KUN).

9b. Ainsliaea elegans Hemsl. var. **strigosa** Mattf., Notizbl. Bot. Gart. Berlin-Dahlem 11(102): 107. 1931. TYPE: China. Yunnan: Mengtze, 8000 ft., *A. Henry 10891* (lectotype, designated here, MO!; duplicate, NY!). Figure 22.

Stem leafy. Leaves concolorous, strigose on both surfaces (with long straight trichomes); cauline leaves similar to the basal, shortly petiolate, gradually reduced upward to the base of the inflorescence.

Distribution and phenology. Southern China (Yunnan) and Vietnam (Chapa) in forest. It was collected at higher elevations than variety *elegans*, between 1800 and 2500 m (vs. 1150–1900 m); flowering in February.

Discussion. The protologue of this taxon includes two specimens. The first was collected at "Yunnan, Mengtze, *A. Henry 10891*" (China) and the second at "Tonkin, Chapa, 1800 m, Feb. 1930, *A. Pételot 3724*" (Vietnam). Even though both specimens totally agree with the protologue, I selected as the lectotype of *Ainsliaea elegans* var. *strigosa* the specimen collected by Henry at Yunnan, deposited at MO, which presents the most complete plant.

Relationships. *Ainsliaea elegans* var. *strigosa* is distinct from *A. elegans* var. *elegans* by its blade abaxial suface strigose (vs. tomentose).

Representative specimen examined. VIETNAM. **Chapa:** Tonkin, *A. Pételot 3724* (paratypes, C, NY, US).

10. Ainsliaea foliosa Hand.-Mazz., Acta Horti Gothob. 12: 348. 1938. TYPE: China. [Sichuan ?]: Sikang, "inter Taining (Ngata) et Taofu (Dawo), Sunglingku, in silva abietina primaeva," 3700 m, 13 Sep. 1934, *H. Smith 12120* (holotype, W!; isotypes, MO!, S!). Figure 23.

Plants perennial, herbaceous, 0.45–0.80 m tall; stems erect, unbranched, leafy, loosely lanuginose. Leaves alternate, approximate above the base, long-petiolate, petioles winged, 4–5.5 cm long, becoming gradually reduced, sessile and distantly spaced upward to the capitulescence; leaf blades chartaceous, ovate to elliptic, 5–8 × 2–3 cm, acute at the apex, base rounded abruptly to slightly narrowly into the petiole, margins obscurely denticulate; slightly discolored, upper surface green-olivaceous, subglabrous, lower surface paler, laxly lanate with long crisped trichomes and glandular biseriate trichomes or with long straight trichomes only; palmati-pinnate, 3-nerved. Capitula sessile, arranged in a stachyoid inflorescence; involucre 4- or 5-seriate, cylindrical, ca. 10 × 3.5 mm; phyllaries papyraceous, glabrous, or sparsely pilose, purple; outer phyllaries ovate, acute, 2–3 × ca. 1.2 mm; inner phyllaries ovate to lanceolate, acute, usually midvein dark, ca. 10 × 0.8 mm. Florets ca. 4, hermaphrodite, chasmogamous and cleistogamous (at different seasons); *chasmogamous florets* with corolla pink, ligulate, 8–9 mm, lobes 3.5–4 mm (deeper slit 4.5–5 mm), tube 3.5–4 mm; anthers ca. 4 mm, apical appendages obtuse, ca. 0.7 mm, sagittate; tails 1–1.5 mm, shortly pilose; style branches truncate, ca. 0.4 mm. Achenes ca. 2 mm, densely pilose, 9-nerved; pappus absent; *cleistogamous florets* with corolla purplish above, tubulose to pseudoligulate, ca. 5 mm, minutely 5-lobed; anthers much reduced, ca. 0.5 mm; style branches ca. 0.7 mm. Achenes ca. 4 mm; pappus bristles ca. 30, ca. 5 mm.

Distribution and phenology. China (Yunnan) in woods between 2000 and 3700 m. The type collection may be from Sichuan; collected in flower in September and April, in flower bud in August.

Relationships. *Ainsliaea foliosa* resembles *A. latifolia* in leaves and capitulescence. Both species have leaf blades ovate to elliptic, long-petiolate, petioles winged. The blade surfaces are lanuginose with long crisped trichomes, and the capitula are arranged in spikes. *Ainsliaea foliosa* may be distinguished by its leaves appearing above the base of the stem (vs. a basal rosette).

Representative specimens examined. CHINA. **Yunnan:** Qiubai Co., 6 May 1952, s. coll. (HITBC 076266); Xujiaba, Jingdong Co., 28 Apr. 1981, s. coll. (HITBC 071881); Langping Co., 2 Aug. 1976, s. coll. (HITBC 076259).

11. Ainsliaea fragrans Champ. ex Benth., Hooker's J. Bot. Kew Gard. Misc. 4: 236. 1852. TYPE: China. Guangdong: Hong Kong, s.d., *J. C. Champion 161* (holotype, K!; isotype, K!). Figure 24.

Ainsliaea rubrifolia Franch., J. Bot. (Morat) 8: 296. 1894. Syn. nov. TYPE: China. Sichuan: Su-tchuen, Tchen-

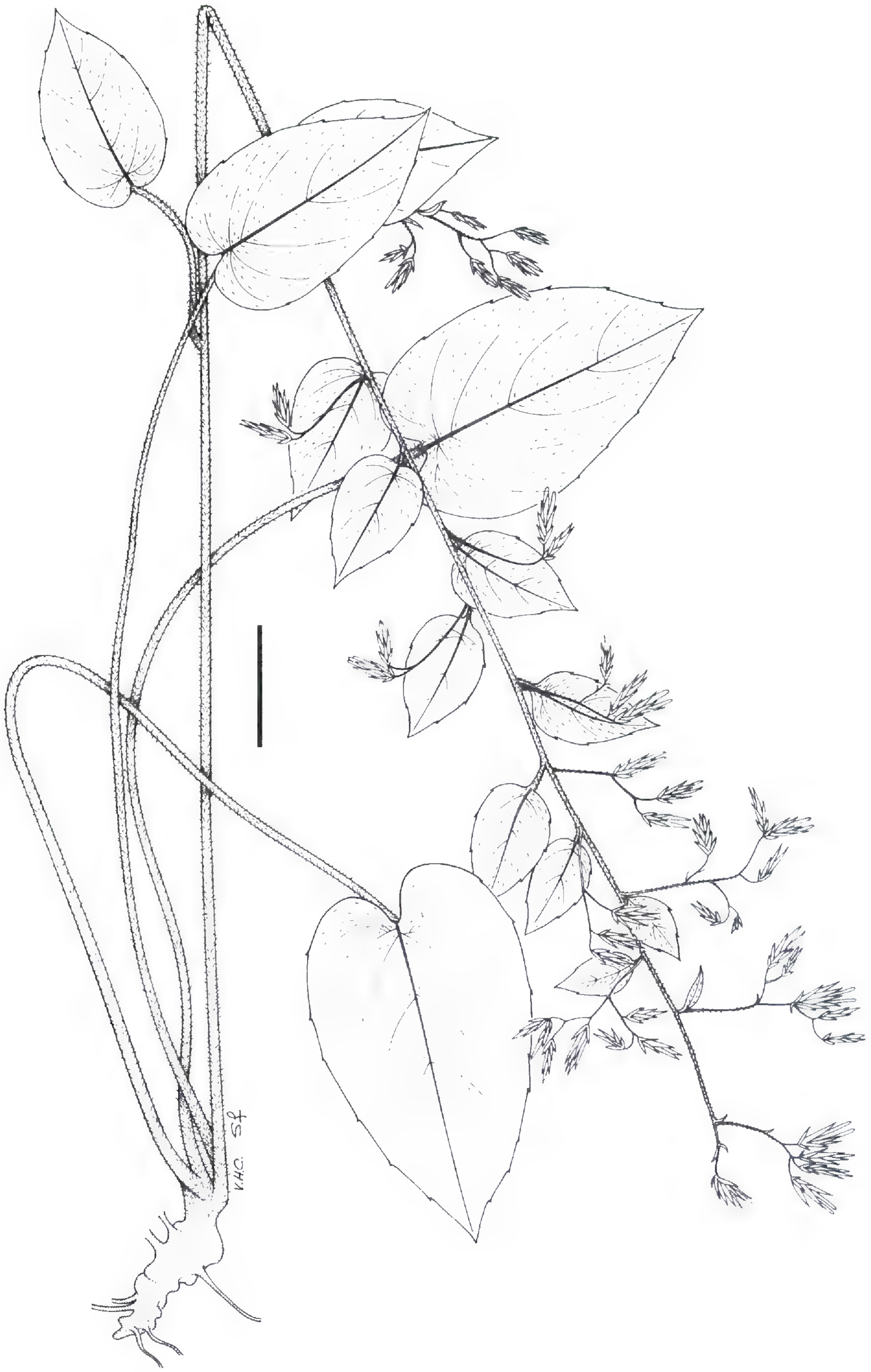


Figure 22. *Ainsliaea elegans* var. *strigosa* Mattf. Scale bar = 3 cm. Drawn from *Henry 10891* (NY).

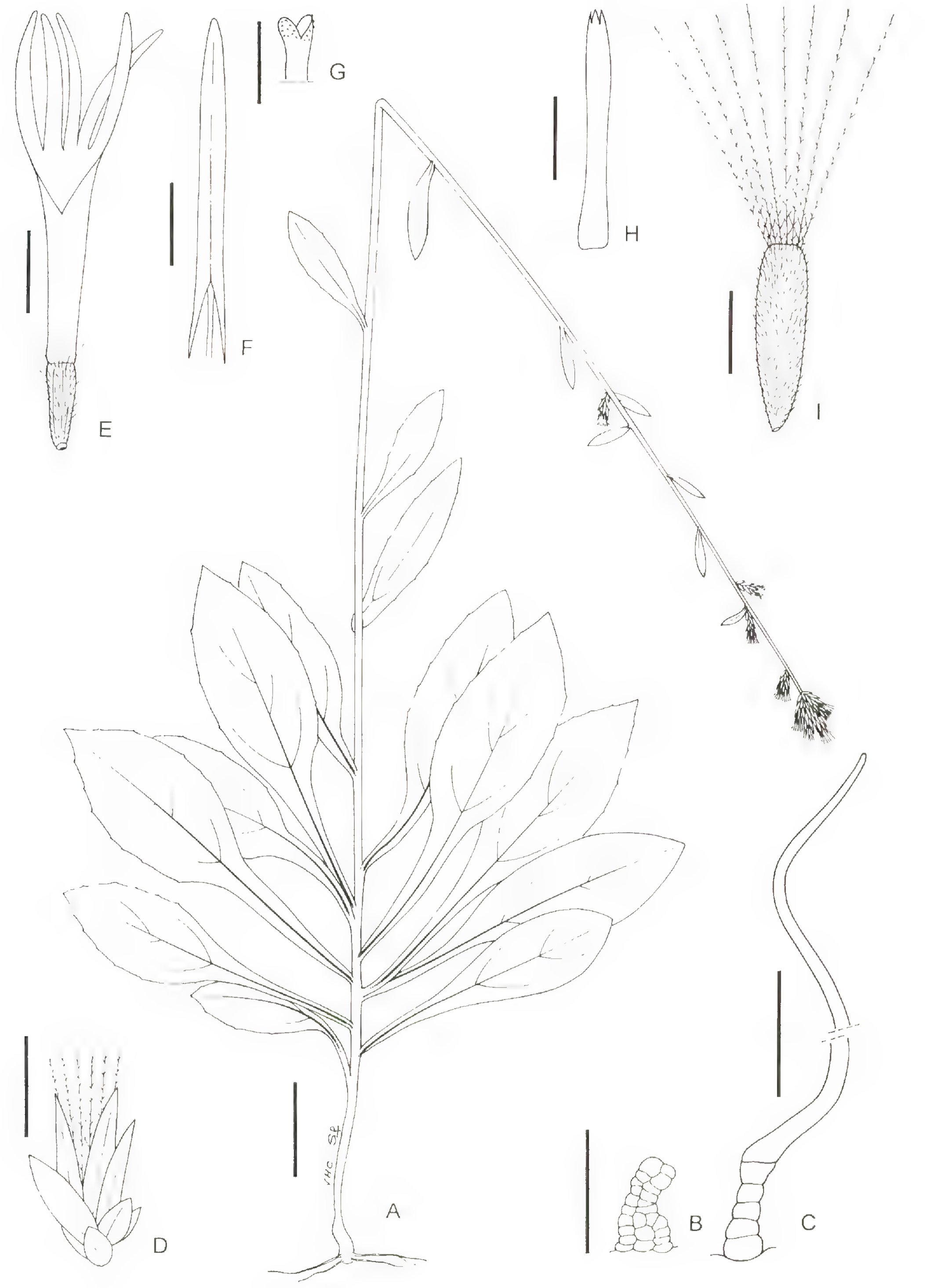


Figure 23. *Ainsliaea foliosa* Hand.-Mazz. —A. Habit. —B, C. Foliar trichomes. —D. Capitulum. —E. Chasmogamous floret (without stamens and style). —F. Stamen of chasmogamous floret. —G. Style branches of chasmogamous floret. —H. Corolla of cleistogamous floret. —I. Achene with pappus of cleistogamous floret. Scale bars: A = 3 cm; B, C = 0.1 mm; D = 5 mm; E, H, I = 2 mm; F, G = 1 mm. Drawn from: A–D, H, I, the isotype *Smith 12120* (MO); E–G, (HITBC 071881).

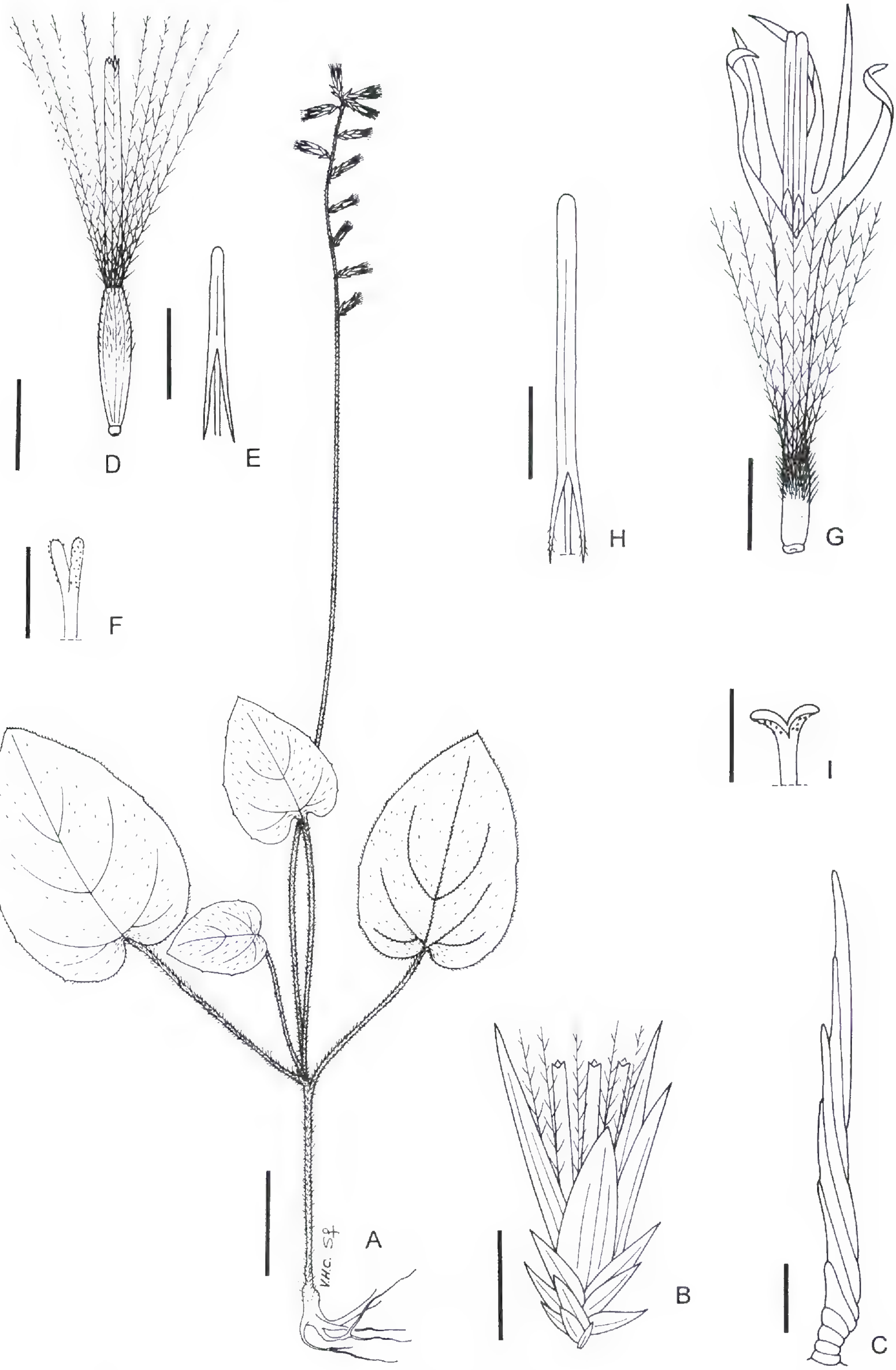


Figure 24. *Ainsliaea fragrans* Champ. ex. Benth. —A. Habit. —B. Capitulum. —C. Foliar trichome. —D. Cleistogamous floret. —E. Stamen of cleistogamous floret. —F. Style branches of cleistogamous floret. —G. Chasmogamous floret. —H. Stamen of chasmogamous floret. —I. Style branches of chasmogamous floret. Scale bars: A = 3 cm; B = 3 mm; C = 0.2 mm; D = 5 mm; E, F = 1 mm; G, I = 1 mm; H = 2 mm. Drawn from: A–C, G–I, *Faber in 1885/86* (S); D–F, *Chow 1978* (NY).

kéou-tin, *R. P. Farges 1034 bis* (holotype, P not seen; isotype, P!)

Ainsliaea cordifolia Franch. & Savat. var. *integrifolia* Maxim., Bot. Jahrb. Syst. 6: 69. 1885. *Ainsliaea integrifolia* (Maxim.) Makino, Bot. Mag. (Tokyo) 22: 167. 1908. *Ainsliaea fragrans* var. *integrifolia* (Maxim.) Kitam., Acta Phytotax. Geobot. 8(1): 67. 1939. Syn. nov. TYPE: Japan. Kyushu: Kumamoto, Higo, Kuma, Pueblo Menda, 25 Nov. 1910, *Y. Narita s.n.* (neotype, designated here, MAK 247989!).

Ainsliaea ningpoensis Matsuda, Bot. Mag. (Tokyo) 27: 236. 1913. Syn. nov. TYPE: China. Zhejiang: Ning-po, *Chang 80* (holotype, TI not seen; isotype, TI photo LP!)

Ainsliaea asarifolia Hayata, Icon. Pl. Formos. 8: 71. 1919. Syn. nov. TYPE: Taiwan. Kelung, Apr. 1916, *B. Hayata s.n.* (holotype, TI not seen; isotype, TI photo LP!).

Plants perennial, herbaceous, 0.17–0.75 m tall; stems erect, simple, villose, naked, with few leaves reduced. Leaves clustered, 1–4(9) cm above stem base, long-petiolate with petioles wingless, strigose, 1–10(14) cm long; leaf blades subcoriaceous, ovate to oblong, 3–11.5(15) × 2–5.5(8) cm, acute to subobtuse at the apex, base deeply cordate, margins obscurely denticulate, with upper surface sparsely strigose to subglabrous, and lower surface commonly purple, red-strigose, especially on the nerves (with long straight trichomes ca. 1.5 mm); palmati-pinnate, 3- to 5-nerved. Capitula sessile or shortly pedunculate, arranged in a stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, 9–13 × ca. 4 mm; phyllaries papyraceous, purple, glabrous; outer phyllaries ovate, acute, ca. 1.5 × ca. 0.5 mm; inner phyllaries linear to elliptic, acute, 3- to 5-nerved, 9–13 × ca. 1.5 mm. Florets fragrant, ♂, hermaphrodite, chasmogamous and cleistogamous (at different seasons or in single plant), corollas white; *chasmogamous florets* with corollas ligulate, ca. 10 mm, lobes ca. 4 mm (deeper slit ca. 5 mm), tube ca. 5 mm; anthers purple, ca. 4 mm, apical anther appendages emarginate, 0.7–1 mm, sagittate; tails 1–1.5 mm, shortly pilose; style branches short, truncate, ca. 0.3 mm. Achenes oblong, ca. 2 mm, 10-nerved, commonly pilose above; pappus bristles 26 to 32, ca. 7 mm, white; *cleistogamous florets* with corollas tubulose to pseudoligulate, 5.5–8 mm, 5-lobed, lobes 0.2–0.4 mm (deeper slit ca. 0.8 mm); anthers ca. 2 mm; style branches 0.5–0.6 mm. Achenes 4–5 mm; pappus bristles 9–10 mm.

Distribution and phenology. Central and southern China (Anhui, Fujian, Guangdong, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Zhejiang), Japan (Kyushu), and Taiwan. It was collected from a rice terrace, from roadside, in very damp shaded ravines, and in pine forest between 0 and 1300 m (Fig. 4A); flowering from August to December.

Discussion. The type designated in the protologue of *Ainsliaea cordifolia* var. *integrifolia* Maxim., "*Kiusiu, Osumi (Tashiro),*" has not been located at KYO, TI, or MAK, no illustration was published with the name, and no other original material has been found. The specimen "*Y. Narita in 1910*" kept at MAK, which is in accordance with the protologue and matches the locality (Higo) indicated in the protologue of *A. integrifolia* (Maxim.) Makino, is here selected as the neotype.

Ainsliaea asarifolia, *A. integrifolia*, *A. ningpoensis*, and *A. rubrifolia* are proposed here as new synonyms of *A. fragrans* because there is no diagnostic character to separate them as distinct taxa. All of these have petiolate leaves that are ovate to oblong, cordate at the base, with margins remotely callose-denticulate and lanuginose underneath, especially on the veins. In addition, the differential character mentioned in the protologue of *A. rubrifolia*, i.e., leaves reddish green on the abaxial surface, is a variable feature throughout the range of *A. fragrans*.

Relationships. *Ainsliaea fragrans* most resembles the species complex of *A. caveleriei*, *A. ramosa*, and *A. rubrinervis*. This complex and *A. fragrans* share leaves that are ovate to oblong, deeply cordate at the base, and villose petioles. *Ainsliaea fragrans* may be distinguished by its rounded apical anther appendages and its leaves in a whorl near the stem base. Remaining species in this species complex have apiculate anther appendages and leaves in basal rosettes.

Vernacular name. Maruba-teishōsō (Makino, 1908; Kitamura, 1965).

Representative specimens examined. CHINA. **Anhui:** "Anhwei," Wu Yuan, Chang Gou Shan [Guoyang], *R. C. Ching 4463* (NY, UC). **Fujian:** *H. Chen 2190* (MO); Yenping, "Cha-ping" [Zhanping], *H. Chung 2906* (UC, W); Kushan Monastery, *S. G. Tang 5788* (UC). **Guangdong:** Canton, *B. Chen et al. 80* (MO); Youle Co., Changxi village on the way to Dadong, *N. G. Chen 42854* (MO); Canton, *China Germany Team 1024* (MO); Luofu Mtn., Huanglong-guan, *L. Deng 347* (MO); Canton, *L. Deng 7048* (MO); Canton, *L. Deng 8530* (MO); Hong Kong, *E. Faber 1885/1886* (C, S); Canton, *Z. Feng 600* (MO); Hong Kong, *C. Ford s.n.* (NY); Guangdong?, *X. P. Gao 50833* (MO); Qixian County, Qujiang, *X. P. Gao 51997* (MO); Canton, *X. P. Gao 53649* (MO); Canton, *Guangdong 77 Team-herb. 5544* (MO); Hong Kong, *Hance 240* (W); Lofaushan, *A. Henry in 1892* (NY); Canton, *H. M. Huang 111593* (MO); Lianping Xian, *Y. K. Lau 111* (AAU); Canton, *Z. Li 716* (MO); Canton, *Z. Li 2031* (MO); Xiangyi Co., *B. Z. Liang? 83655* (MO); Lofaushan, *E. D. Merrill 10230* (US); Canton, *P. X. Tan 59543* (MO); Lian Xian, *P. X. Tan 59863* (MO); Canton, *P. X. Tan 60508* (MO); "Kwangtung," Loh Ch'ang District, Hung Pui Ts'oi, Chong Uen Shan near Kau Fung, *W. T. Tsang 20812* (MO, NY, UC, US); "Kwangtung," Chung Tung, Tsi Tsan, Ying Tak, *W. T. Tsang & K. C. Wong 3177, herb. 15038* (UC); "Ruyuan Hsien" [Ruyuan Xian], *C. Wang 44058* (MO), *C. Wang 44094* (MO); Lofaushan, no further data, s. coll. (MO 3935534). **Hubei:** "Hupeh," *H. C. Chow 1978* (NY);

"Hupeh," *Y. X. Chong 3882* (CM). **Hunan:** Ma-Ling-Tung, *C. S. Fan & Y. Y. Li 620* (W). **Jiangsu:** "Jiangsu," I-hing, *W. Z. Fang 8017* (CM, MO); "Kiangsu," Chinkiang, *K. Kollhoff 218* (W); **Jiangxi:** Xiu-shiu Co., "Jiangsi," *S. L. Liu 890169* (MO). **Sichuan:** "Szechuan," Omei Hsien, Mt. Omei, *C. L. Chow 4909* (US); Mt. Omei, *Y. S. Liu 1748* (US). **Without locality & collector:** 25 Nov. 1910 (MAK 247989), Aug. 1910 (MAK 157210), 2 Oct. 1910 (MAK 247990). JAPAN. Kyushu. Oshumi, Higashisonoyamamura, *S. Hatusima 16515* (US). **Miyazaki:** Hiyuga, *Z. Tashiro in 1910* (MAK 157210); Hiyuga, Miyazaki-1.2, 26 Nov 1912, s. coll. (MAK 11690). **Kagoshima:** Satsuma, Pueblo Yoshino, *B. Fukasawa in 1910* (MAK 247990).

12. Ainsliaea fulvipes Jeffrey & W. W. Sm., Notes Roy. Bot. Gard. Edinburgh 8: 175. 1914. TYPE: China. Yunnan: Teng-yueh 25°15′, 7000 ft., May 1912, *G. Forrest 7862* (lectotype, designated here, S!). Figure 25.

Plants perennial, herbaceous, 0.15–0.45 m tall; stems erect, unbranched, naked, with few leaves only, much reduced, strigose. Leaves basally clustered, rosulate, long-petiolate; petioles wingless, 2–7 cm, densely strigose; leaf blades subcoriaceous, oblong to ovate or oblong to elliptic, 3–7.5 × 1.5–4 cm, apex rounded to subobtuse, base rounded, margins shallowly denticulate and strigose; strigose on both surfaces, with straight trichomes, 1–1.8 mm; palmati-pinnate, 3-nerved. Capitula sessile or shortly pedunculate, arranged in a stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, 7–10 × 2–3 mm; phyllaries subcoriaceous, purplish and pilose above; outer phyllaries ovate, acute, 1.5–2.5 × ca. 1 mm; inner phyllaries linear to elliptic, acute, midvein dark, ca. 10 × 1 mm. Florets 3, hermaphrodite, cleistogamous; corollas pale rose, tubulose to pseudoligulate, ca. 3.5 mm, 5-lobed, lobes ca. 0.3 mm (deeper slit ca. 0.5 mm); anthers ca. 1.8 mm, apical anther appendages rounded, ca. 0.25 mm, sagittate; tails ca. 0.7 m, slightly pilose; style branches short, truncate, ca. 0.4 mm. Achenes oblong, 3–3.5 mm, 10-nerved, densely pilose; pappus bristles 28 to 30, ca. 5.5 mm.

Distribution and phenology. *Ainsliaea fulvipes* is known only from southern and central China (Guangdong, Sichuan, Yunnan), collected from mixed forest on rocks, between 2100 and 2600 m; flowering in May and November, flower bud in September and October.

Discussion. The protologue of *Ainsliaea fulvipes* includes two specimens from the same locality, "Teng-yueh, *Howell 252*" and "Teng-yueh, *G. Forrest 7862*," the former matched as "type." Because I was unable to locate the type collection and no other original material has been found, I selected as lectotype of this taxon the specimen "*G. Forrest 7862*" kept at S.

Relationships. *Ainsliaea fulvipes* most resembles *A. angustata* from Sichuan province in China. Both species have as leaves basal rosettes that are long-petiolate with these petioles densely strigose. *Ainsliaea fulvipes* may be differentiated by its leaf blades oblong to elliptic (vs. linear to elliptic), with obtuse to rounded apex (vs. acute), phyllaries pilose above (vs. glabrous), and rounded anther appendages (vs. acuminate).

Representative specimens examined. CHINA. **Guandong:** Guangzhou [Canton], *X. Liu 24979* (MO). **Sichuan:** Kansei, *S. Q. Chen 15486* (MO). **Yunnan:** Betw. Tengyueh & the Burmese border, rd. to Sadon, near Lin Chi, *J. F. Rock 7284* (US, W); Luqian Co. Xiaohou street, *Y. B. Zhang 0283* (HITBC); Tengchong Co., Yunhua, *780 Team No. 833* (HITBC).

13. Ainsliaea glabra Hemsl., J. Linn. Soc., Bot. 23: 171. 1888. TYPE: China. Sichuan: Szechuan, Mt. Omei, 4000 ft., Dec. 1887. *E. Faber 391* (lectotype, designated here, K!; duplicate, NY!). Figure 26.

Ainsliaea lancifolia Franch., Nouv. Arch. Mus. Hist. Nat., Ser. 2. 10: 41. 1888. Syn nov. TYPE: China. Xizang: Tibet Oriental, Prov. Moupin, Apr. 1869. *M. l'Abbé David s.n.* (holotype, P not seen; isotypes, P photo LP!, P!).

Plants perennial, herbaceous, 0.25–1.50 m tall; stems erect, unbranched, leafy, loosely strigose. Leaves basally clustered, rosulate, long-petiolate with petioles wingless, petioles 5–13(18) cm, flat; leaf blades slightly fleshy, ovate or ovate to elliptic, 6–15(20) × 2–6.5(9) cm, apex acute to acuminate, base longly cuneate into the petiole, margins obscurely denticulate; upper surface subglabrous with scarce, long straight trichomes and lower surface paler, glabrous; palmati-pinnate, 3-nerved, veins purple on the lower surface; cauline leaves alternate, sessile, ovate to lanceolate, ca. 2.5 × 1 cm. Capitula pedunculate, arranged in thyrsoid inflorescence; peduncles with glandular trichomes; involucre 5- or 6-seriate, cylindrical, 6–8 × 2–5 mm; phyllaries purple, papyraceous; outer phyllaries broadly ovate, acute, ca. 1 × 0.5 mm, glabrous or sparsely pilose; inner phyllaries linear to elliptic, subacute, midvein dark, ca. 6 × 1 mm, glabrous (rarely with short glandular trichomes). Florets 3, hermaphrodite, cleistogamous and chasmogamous (at different or same seasons); corollas white or pink; *chasmogamous florets* with corolla ligulate, ca. 8 mm, lobes ca. 4 mm (deeper slit ca. 5 mm), tube ca. 3 mm; anthers ca. 4 mm, apical appendages truncate, 0.3–0.4 mm, sagittate; tails 0.8–0.9 mm, shortly pilose; style branches truncated, ca. 0.5 mm. Achenes linear-elliptic, ca. 2.5 mm, 10-nerved; pappus bristles ca.

Figure 25. *Ainsliaea fulvipes* Jeffrey & W. W. Sm. —A. Habit. —B. Foliar trichome. —C. Capitulum. –D. Phyllaries. —E. Cleistogamous floret. F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 0.1 mm, C = 5 mm; D = 2 mm; E = 1.5 mm; F, G = 1 mm. Drawn from A, the paratype *Forrest 7862* (S); B–G, *Rock 7284* (US).

38, 5–6 mm; *cleistogamous florets* with corolla tubulose to pseudoligulate, 3.5–7 mm, 5-lobed, lobes ca. 0.2 mm (deeper slit ca. 0.5 mm), anthers ca. 2 mm; style branches ca. 0.5 mm. Achenes 3–4 mm; pappus bristles ca. 7.5 mm.

Distribution. China (Guizhou, Hubei, Hunan, Sichuan, Xizang, Yunnan) (Fig. 5B).

Discussion. Hemsley (1888: 471) mentioned in the protologue the collection "*Faber!*". Although I was unable to find this specimen, I located at K and NY the collection "*Faber 391*," which totally corresponds to the protologue in its detail, including agreement for the date, and consequently, I propose the specimen kept at K as the lectotype because, according to Stafleu and Cowan (1979), Hemsley's materials can be found in this herbarium.

Ainsliaea lancifolia is proposed as new synonym of *A. glabra* because there is no diagnostic character that differentiates the former from the latter.

Relationships. *Ainsliaea glabra* is closely related to *A. nervosa.* Both species have subglabrous leaves that are long-petiolate, with wingless petioles. *Ainsliaea glabra* may be distinguished by its slightly fleshy, ovate to elliptic leaf blades (vs. narrowly elliptic and subcoriaceous).

Key to the varieties of Ainsliaea glabra *in China*

1a. Achenes glabrous or with few trichomes above . . . . . 13a. var. *glabra*
1b. Achenes densely pilose . . . . . 13b. var. *sutchuenensis*

13a. Ainsliaea glabra Hemsl. var. **glabra**. Figure 26A–L.

Florets cleistogamous or chasmogamous with corollas white or light purple. Achenes glabrous or apically pilose.

Distribution and phenology. China (Guizhou, Sichuan, Xizang, Yunnan), from the edge of woods and on grassy slopes, between 1000 and 2000 m; collected in flower in April and July, in fruit in May and September, in flower bud in October.

Representative specimens examined. CHINA. **Guizhou:** "Kweichow," Tungtze, *Y. Tsiang 5087* (NY). **Sichuan:** Paohsing-hsien, *K. L. Chu 3939* (W); Kuan Hsien, *W. P. Fang 1990* (US), *W. P. Fang 1996* (US, W); Omei Hsien, *W. P. Fang 3157* (W); Ku-long-tchang, *E. E. Maire s.n.* (W7225); Omei Hsien, Mt. Omei [Emei], *W. H. Wang 8078* (US). **Yunnan:** Beyendjing, Tsin-choei-ky, *P. S. Ten 354* (C, W).

13b. Ainsliaea glabra Hemsl. var. **sutchuenensis** (Franch.) S. E. Freire, comb. et stat. nov. Basionym: *Ainsliaea sutchuenensis* Franch., J. Bot. (Morot) 7: 296. 1894. TYPE: China. Sichuan: "Su-tchuen oriental, Tchen-keou-tin, Ky-min-sé près de Tcheu Keou," 1200 m, 3 May 1892, *R. P. Farges 1034B* (holotype, P not seen; isotypes, P photo LP!, P!). Figure 26M.

Ainsliaea tenuicaulis Mattf., Notizbl. Bot. Gart. Berlin-Dahlem 11(102): 108. 1931. *Ainsliaea glabra* var. *tenuicaulis* (Mattf.) C. C. Chang, Bull. Fan. Mem. Inst. Biol., Bot. 5: 319. 1934. Syn. nov. TYPE: China. Hubei: W. Hupeh, Apr. 1901, *E. H. Wilson 1766* (lectotype, designated here, W!; duplicate, NY!).

Ainsliaea plantaginifolia Mattf., Notizbl. Bot. Gart. Berlin-Dahlem 11(102): 107. 1931. Syn. nov. TYPE: China. Hunan: Angabe, Aug. 1926, *S. S. Sin 181* (lectotype, designated here, W!).

Florets chasmogamous with corollas pink. Achenes densely pilose.

Distribution and phenology. China (Hubei, Hunan, Sichuan), growing at forest margins and on grassy slopes, between 720 and 1300 m; collected in flower in April, in fruit in May.

Discussion. *Ainsliaea glabra* var. *sutchuenensis* is given varietal status because it possesses densely pilose achenes; otherwise it is typical of the species.

Ainsliaea tenuicaulis and *A. plantaginifolia* are proposed as new synonyms of *A. sutchuenensis* (= *A. glabra* var. *sutchuenensis*) because there is no diagnostic character to separate them as distinct taxa.

Because the collection mentioned in the protologue of *Ainsliaea tenuicaulis*, "*E.H. Wilson* n. *1766, Hb. Berol.*," was probably destroyed, the collection deposited at W is selected as lectotype because it presents the most complete plant. The holotype specimen of *A. plantaginifolia* at B was also probably destroyed, and, therefore, the isotype at W, which is sufficiently diagnostic, is designated as lectotype.

Representative specimens examined. CHINA. **Sichuan:** "Su-tchuen oriental, Tcheu-kéou-tin" [Chengkou], s.d., *R. P. Farges s.n.* (US); Nanchuan Co., *Z. Y. Liu 15451* (CANB); Omei Hsien, Mount Omei, *C. L. Sun 1462* (US). **Hubei:** Changyang (Chang-Jiang), *A. Henry 7599* (paratype of *A. tenuicaulis*, MO).

14. Ainsliaea glumacea (Fr.) Sch. Bip., Jahresber. Pollichia 20-21: 407. 1863. Basionym: *Hieracium glumaceum* Fr., Epicr. Hierac.: 141. 1861(1862). TYPE: India, Silhet, year 1830, *Wallich s.n.* (lectotype, designated here, K-WALL 3280 not seen; duplicate, K photo LP!). Figure 27.

Ainsliaea angustifolia Hook. f. & Thoms. ex C. B. Clarke, J. Linn. Soc. 14: 412. 1875. Syn. nov. TYPE: India. Khasia, 4000–6000 ped., s.d., *J. D. Hooker & T. Thomson s.n.* (holotype, K!; isotypes, C!, M!, NY!, S!, UC!, US!, W!).

Figure 26. *Ainsliaea glabra* Hemsl. A–L. *A. glabra* var. *glabra*. —A. Habit. —B, C. Foliar trichomes. —D. Phyllaries. —E. Capitulum. —F–H. Cleistogamous floret: F. corolla; G. stamen; H. style. —I–L. Chasmogamous floret: I. stamen; J. style branches; K. corolla; L. achene with pappus. —M. *A. glabra* var. *sutchuenensis* (Franch.) S. E. Freire. Achene with pappus of chasmogamous floret. Scale bars: A = 3 cm; B = 0.1 mm; C = 0.05 mm; D, I, L, M = 2 mm; E, K = 5 mm; F–H, J = 1 mm. Drawn from A, *David in 1870* (P); B–H, M, *Fang 1996* (US); I–K, *Ten 354* (W); L, *Wilson 1766* (NY).

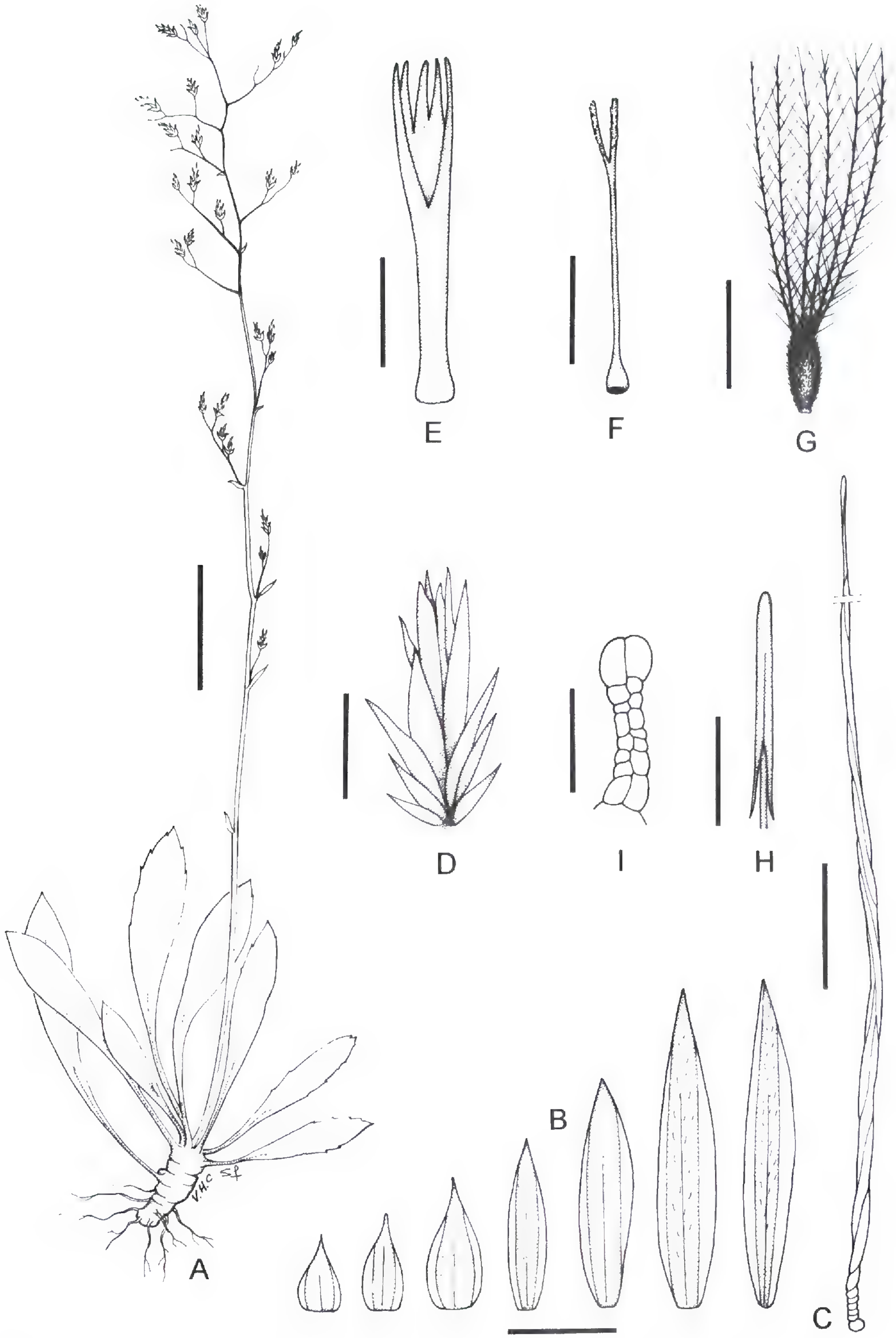


Figure 27. *Ainsliaea glumacea* (Fr.) Sch. Bip. —A. Habit. —B. Phyllaries. —C. Foliar trichome. —D. Capitulum. —E–H. Cleistogamous floret: E, corolla; F, style; G, achene with pappus; H, stamen. —I. Glandular trichome of inner phyllary. Scale bars: A = 3 cm; B, D, G = 2 mm; C = 0.3 mm; E, F = 1 mm; H = 0.5 mm; I = 0.05 mm. Drawn from *Hooker f. & Thomson s.n.* (US).

Plants perennial, herbaceous, (0.14)0.25–0.35 m tall: stems erect, unbranched below the inflorescence, with few leaves much reduced, moderately strigose. Leaves basally clustered, rosulate, sessile; leaf blades slightly fleshy, linear to obovate, 6–10 × 1–1.5 cm, apex acute to obtuse, longly attenuate at the base; margins obscurely denticulate above; upper surface sparsely strigose to glabrous and lower surface sparsely strigose, with long straight trichomes, 2–2.5 mm; palmati-pinnate, 3-nerved. Capitula pedunculate, arranged in thyrsoid inflorescence; involucre 5- to 7-seriate, cylindrical, 5–9 × 1.5–3(5) mm; phyllaries papyraceous; outer phyllaries ovate to lanceolate, acuminate, 1.5–2.8 × 0.8–1.5 mm, glabrous; inner phyllaries narrowly lanceolate, acute, midvein dark, 6.5–7.5 × 1–1.2 mm, shortly pubescent (glandular and non-glandular trichomes). Florets 3 or 4, hermaphrodite, cleistogamous; corollas white or pink, tubulose to pseudoligulate, 2.8–3.7 mm, lobes 0.25 0.75 mm (deeper slit 1–1.5 mm), tube 1.8–2.2 mm; anthers ca. 1.5–1.7 mm, apical appendages rounded, 0.4–0.5 mm, sagittate; tails 0.5–0.7 mm, smooth to slightly pilose; style branches short, rounded, 0.6–1 mm. Achenes oblong, 1.5–3 mm, 9-nerved, densely pilose; pappus bristles 25 to 30, ca. 5 mm.

Distribution and phenology. Bangladesh, India, and Pakistan, collected from moist, shady places along river and stream margins amid rocky boulders, between 600 and 1500 m (Fig. 5A); flowering in February and March and from October to December.

Discussion. The name *Ainsliaea angustifolia*, which appeared as a correct name in the Index Kewensis, was described in 1875 by C. B. Clarke from the Hooker f. & Thomson collection from Khasya (India). One year later, Clarke used this name in his survey of *Compositae Indicae* (1876: 247). However, 13 years earlier in 1862, E. M. Fries had published *Hieracium glumaceum* from Silhet (India) in Epicr. Hierac. (= Upsala Univ. Arsskr.), which was transferred to the genus *Ainsliaea* by Schultz Bipontinus in 1863. Both description and transference were overlooked by Clarke, perhaps because of the unexpected places of publication. The name *A. angustifolia* was also used by J. D. Hooker in his *Flora of British India*. Although he referred in his treatment to the name *A. glumacea*, his reference is to the species described by Klatt in 1878 (Sitzungsb. Munch. Kad.: 97), not to *A. glumacea* (D. Don) Sch. Bip., which was obviously placed in synonymy. The name *Hieracium glumaceum* Fr. was published by E. M. Fries based on a Wallich specimen, without mentioning details of the collection, although there is some suspicion that the specimen was *Wallich Cat. 3280*. In fact, Fries mentioned in his description that the specimen on which he based *H. glumaceum* was widely distributed under the unpublished manuscript name of Prenanthes candolleana Wall. (= *Wall. Cat. 3280*), in his words "cum priori s.n. Prenanth. Candollei promiscue distribuit *Wallich* ...." Furthermore, when Schultz Bipontinus (1863: 407–408) transferred *H. glumaceum* to the genus *Ainsliaea*, he mentioned this specimen ("Wallich 3280 ex parte").

Wallich 3280 seems to be a problematic specimen, i.e., as pointed out by Clarke (1875: 411), "an original sheet in the Calcuta-herbarium of Wallich, numbered 3280, with a reference to the comp. N.390, which number refers to the name Prenanthes candolleana Wall., corresponds to the description of *Hieracium silhetense* DC." (it was seen by references on the same sheet by de Candolle and Fries!). Furthermore, Clarke (1875: 413) also says that another "authentic specimen of *Hieracium silhetense* (Wallich 3280 with the number probably written on it in Wallich's own hand) is *Ainsliaea angustifolia* Hook. f. et Thoms." In other words, under one Wallichian number and name, two species, *H. silhetense* DC. and *A. angustifolia* Hook. f. & Thoms. ex C. B. Clarke, were mixed. A few years later, one further species is linked to *Wallich 3280*, i.e., Hooker (1881: 388) mentioned "Prenanthes? Candolleana, Wall. Cat. 3280, in part" as a synonym of *A. pteropoda* DC.

Dr. D. J. N. Hind at K sent me a photo with two specimens mounted on it. The first was collected in Silhet, of which the label reads "Prenanthes Candolleana Wall, Monte Silhet Wallich 1830, 3280/390," and the second was collected in Khasya, of which the label reads "Khasya/Griffith/Leman 1845." The specimen collected in Silhet in 1830, which totally agrees with the protologue, is here designated as the lectotype of *Hieracium glumaceum*.

After examining the type material, I consider that there are no diagnostic characters with which to separate *Ainsliaea angustifolia* and *A. glumacea*.

Relationships. *Ainsliaea glumacea* shared a similar habit with *A. latifolia* (D. Don) Sch. Bip. (including var. *taiwanensis*), as a scapose perennial with a basal rosette. *Ainsliaea glumacea* is clearly distinguished by its leaf blades that are linear to obovate (vs. ovate to elliptic), attenuate and narrow at the base (vs. abruptly contracted), and strigose beneath (vs. tomentose), as well as by its smaller capitula (5–9 mm) and achenes (1.5–3 mm) (vs. 8–10 mm and 3.5–5.5 mm, respectively, in *A. latifolia*). This species also resembles *A. reflexa* in its leaves, but *A. glumacea* is distinct from *A. reflexa* by its leaf blades attenuate and sessile (vs. leaves long-petiolate with the blades abruptly contracted and cuneate) as well as by the thyrsoid inflorescence (vs. stachyoid in *A. reflexa*).

Representative specimens examined. BANGLADESH. "East Bengal," *Griffith 3329/1* (C, M, S); "Afghanistan," *Griffith 3329/1* (NY). INDIA. **Assam:** Khasia, *J. D. Hooker s.n.* (NY); Jowai, Jaintia Hills, *Dr. Kings in 1897* (CAL); Cherrapungi, *G. Panigrahi 4798* (CAL); Eastern Circle, Shillong, Subansiri F.D. (N.E.F.A.), Hapoli, *G. Panigrahi 19832* (CAL); Meghalaya, Jing King Swan, Lang Laosi, Nongstoin, *P. C. Parot 51434* (MO); Eastern Circle, Shillong, Subansiri F.D. (N.E.F.A.), Hapoli, *A. R. K. Sastry 41016* (CAL); "Environs of Tserapúndzi till near Máirong," ex herb. *Schlagintweit, Cat. No. 391* (US). PAKISTAN. Prov. Punjab, environs of "Raulpindi" [Rawalpindi], ex herb. *Schlagintweit, Cat. No. 10864* (UC); Prov. Punjab, Kalabagh, ex herb. *Schlagintweit, Cat. No. 11960* (S).

15. Ainsliaea henryi Diels, Bot. Jahrb. Syst. 29: 628. 1901. *Ainsliaea latifolia* subsp. *henryi* (Diels) H. Koyama, Acta Phytotax. Geobot. 32 (1–4): 60. 1981. TYPE: China. Hubei: Hupeh, Mar. 1889, *A. Henry 6639* (type, K!). Figure 28.

Ainsliaea undulata Diels, Bot. Jahrb. Syst. 29: 629. 1901. TYPE: China. Sichuan: S Nan'chuan: Huo pan chu p'ing, *C. Bock et A. v. Rosthorn 773* (type, O!).

Plants perennial, herbaceous, 0.10–0.55 m tall; stems erect, unbranched, naked, loosely strigose. Leaves basally clustered, rosulate; leaf blades membranous, apex acute, margins slightly wavy, obscurely denticulate-mucronate; upper surface subglabrous, and lower surface sparsely strigose with long straight trichomes, ca. 1.5–2 mm; palmati-pinnate, 3-nerved. Capitula sessile or short-pedunculate, arranged in a stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, ca. 7.5 × 2.5 mm; phyllaries papyraceous, apically purple and strigose; outer phyllaries narrowly ovate, acuminate, ca. 2.5 × 1 mm; inner phyllaries linear, acute, midvein dark, ca. 7.5 × 0.6 mm. Florets 1 to 3, hermaphrodite, cleistogamous and chasmogamous without pappus (usually at different seasons); corollas white; *chasmogamous florets* with corollas ligulate, ca. 7.5 mm, lobes 2.5–4 mm (deeper slit ca. 5 mm), tube ca. 2.5 mm; anthers ca. 3.5 mm long, apical appendages rounded, ca. 0.8 mm, sagittate; tails ca. 0.8 mm, pilose; style branches short, truncate, ca. 0.3 mm. Achenes oblong-elliptic, ca. 1.5 mm, glabrous or shortly pilose above, 10-nerved, pappus absent; *cleistogamous florets* with corollas tubulose to pseudoligulate, ca. 3.5 mm, 5-lobed, lobes ca. 0.2 mm (deeper slit ca. 0.3 mm), tube 3.2 mm; anthers ca. 0.7 mm; style branches ca. 0.4 mm. Achenes narrowly oblong, 2.5–5 mm, 10-nerved, glabrous or shortly pilose above; pappus bristles 29 to 35, ca. 6 mm.

Distribution. China (Fujian, Guangxi, Guizhou, Hubei, Hunan, Sichuan, Yunnan) and Taiwan (Fig. 5A).

Discussion. Although Koyama (1981) regarded *Ainsliaea henryi* as a subspecies of *A. latifolia*, I consider that it should be retained as a distinct species. The leaves of typical *A. latifolia* are ovate with winged petioles, the achenes are pubescent, and the inflorescences consist of generally three or four capitula per node. The leaves of *A. henryi* are obovate subsessile to only short-petiolate, the achenes are glabrous or apically pubescent, with the inflorescence as a solitary capitulum or two or three capitula.

Relationships. Ainsliaea henryi resembles *A. reflexa* in leaf shape, ovate to obovate and ovate to elliptic, respectively, but it is distinguished by its achenes glabrous or apically pilose (vs. pilose in *A. reflexa*).

Ainsliaea henryi is recognized in this treatment with two varieties, their distribution overlapping only in Taiwan.

Key to the varieties of Ainsliaea henryi *in China and Taiwan*

1a. Leaves obovate, attenuate into the petiole, petiole winged on the upper half . . . . . 15a. var. *henryi*
1b. Leaves ovate, rounded or cuneate at the base, petiole wingless . . . . . 15b. var. *subalpina*

15a. Ainsliaea henryi Diels var. **henryi**. Figure 28A–L.

Leaves obovate to oblong, 4.5–8.5 × 1.8–3 cm, subobtuse at the apex and attenuate at the base; petiole winged in upper half.

Distribution and phenology. China (Fujian, Guangxi, Guizhou, Hunan, Hubei, Sichuan) and Taiwan, collected in rock crevices and forests, at elevations between 1570 and 1800 m; flowering from July to October and in December, January, and March.

Representative specimens examined. CHINA. **Fujian:** *S. Wuyi Team 80-0203* (MO). **Guangxi:** Northern "Kwangsi" [Shangsi], Kwei-lin District, Hsi-chang village & vicinity Ch'i-fen-shan, Kwei-lin, *W. T. Tsang 28500* (US). **Guizhou:** "S Wushan" [Wuchuan], *E. H. Wilson 2159* (W). **Hubei:** "Hupeh," *A. Henry 7461* (NY); "W Hupeh," *E. H. Wilson 2159* (NY). **Hunan:** Hunan, Xinning, Ziyunshan, *Z. Y. Li et al. 326* (CANB); Hunan, *P. X. Tan 62920* (MO). TAIWAN. **Nan-tou:** Ten-tzu to nen-kao, Co. Nantou, *T. C. Huang et al. 5798* (MO).

15b. Ainsliaea henryi var. **subalpina** (Hand.-Mazz.) S. E. Freire, comb. nov. Basionym: *Ainsliaea reflexa* Merr. var. *subalpina* Hand.-Mazz., Akad. Wiss. Wien, Math.-Naturwiss. Kl., Denkschr. 63(1): 12. 1926. TYPE: China. "Inter urbem Yungbei et pagum Yungning, in montis Hungguwo prope Hsinyingpan, in fruticetis

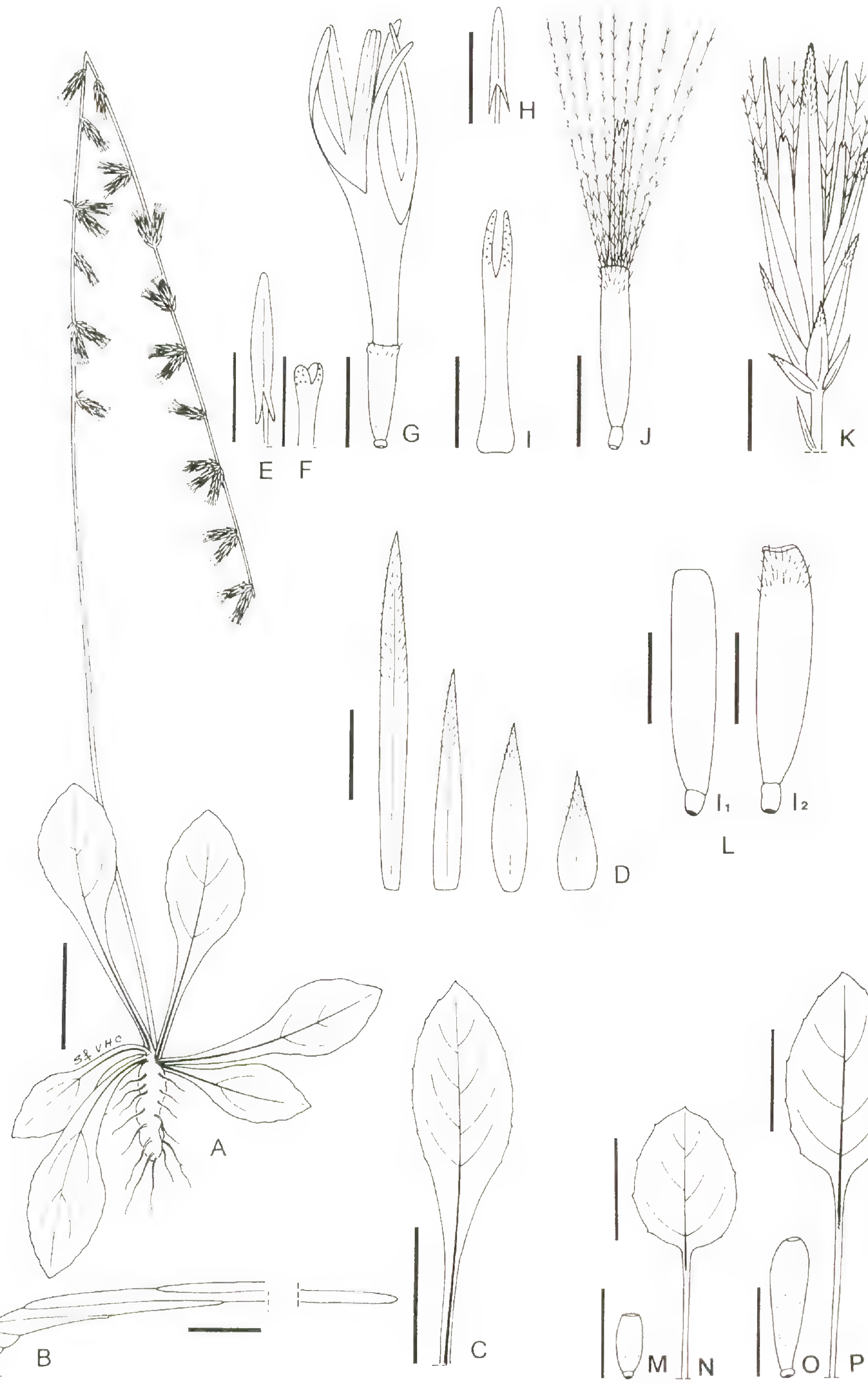


Figure 28. *Ainsliaea henryi* Diels. A–L. *A. henryi* var. *henryi*. —A. Habit. —B. Foliar trichome. —C. Leaf. —D. Phyllaries. —E. Stamen of chasmogamous floret. —F. Style branches of chasmogamous floret. —G. Chasmogamous floret. —H. Stamen of cleistogamous floret. —I. Style of cleistogamous floret. —J. Cleistogamous floret. —K. Capitulum. —L. Achenes of cleistogamous florets. M–P. *A. henryi* var. *subalpina* (Hand.-Mazz.) S. E. Freire. —M. Achene of chasmogamous floret. —O. Achene of cleistogamous floret. —N, P. Leaves. Scale bars: A = 3 cm; B = 0.1 mm; C = 2 cm; D = 2 mm; E, G = 2 mm;

bambusetisque," 3100–3450 m, 28 June 1914, *F. Handel-Mazzetti 3282*, Diar. No. 670 (holotype, W!). Figure 28M–P.

Ainsliaea morrisonicola Hayata, J. Coll. Agric. Imp. Univ. Tokyo 25(19): 142. 1908. Replaced name: *Ainsliaea elegans* Hayata, Bot. Mag. (Tokyo) 20(229): 14. 1906, non *Ainsliaea elegans* Hemsley, Hooker's Icon. Pl. 28(2): t. 2747. 1902. *Ainsliaea reflexa* f. *morrisonicola* (Hayata) Suzuki, Annual Rep. Taihoku Bot. Gard. 1: 180. 1931. *Ainsliaea reflexa* var. *morrisonicola* (Hayata) Yamam., J. Soc. Trop. Agric. 8: 264. 1936. Syn. nov. TYPE: China. Taiwan. Mt. Morrison, Seizan ad 11707 ped, 3 Nov. 1905, *S. Nagasawa, T. Kawakami et G. Nakahara s.n.* (type, PH 51684!).

Ainsliaea reflexa Merr. var. *nimborum* Hand.-Mazz., Akad. Wiss. Wien, Math.-Naturwiss. Kl., Denkschr. 63(1): 12. 1926. *Ainsliaea latifolia* var. *nimborum* (Hand.-Mazz.) Kitam., Acta Phytotax. Geobot. 19(1): 14. 1961. Syn. nov. TYPE: China. Yunnan: In pluviisilvis mixtis temperatis vallis Doyonlumba ad fluvium Lu-djiang (Salween), ca. 28°2′, 3250–3450 m, 23 Sep. 1915, *F. Handel-Mazzetti 8343*, Diar. Nr. 1538 (holotype, W!; isotype, WU!).

Ainsliaea henryi var. *ovatifolia* C. C. Chang, Sinensia 4(8): 227. 1934. Syn. nov. TYPE: China. Yunnan: without locality, *G. Forrest 5681* (holotype, K!; isotype, K photo LP!).

Leaf blades ovate, 2.5–3.8 × 1.5–2.4 cm, rounded or cuneate at the base, with long slender wingless petioles, 3.5–5.5 cm.

Distribution and phenology. China (Yunnan) and Taiwan, from *Abies* Mill. forest, from sunny grassland, and from *Tsuga* (Endl.) Carrière community, from elevations between 2000 and 3900 m; collected in flower in June, August, September, and November.

Discussion. I propose to transfer *Ainsliaea reflexa* var. *subalpina* Hand.-Mazz. from *A. reflexa* to *A. henryi* because it possesses glabrous achenes (vs. pilose in *A. reflexa*); consequently, the new combination *A. henryi* var. *subalpina* (Hand.-Mazz.) S. E. Freire is made.

Ainsliaea reflexa var. *nimborum* was said to differ from *A. reflexa* var. *subalpina* (= *A. henryi* var. *subalpina*) by its achenes without pappus (vs. achenes with pappus in the var. *subalpina*). However, it is a variable feature throughout the range of *A. henryi* var. *subalpina*.

After examining the type material, I consider that there are no diagnostic characters with which to separate *Ainsliaea morrisonicola* and *A. henryi* var. *ovatifolia* from *A. henryi* var. *subalpina*. All have glabrous achenes and ovate leaves that are round or slightly cuneate at the base, with long slender wingless petioles.

Observation. *Ainsliaea hayatae* Beauverd (Bull. Soc. Genève, Sér. 2, 2: 382. 1910) and *A. hayatae* H. Lév. (Cat. Pl. Yunnan 36. 1915), which were proposed to replace *A. elegans* Hayata, represent later homonyms.

Vernacular name. Okada-haguma (Yamamoto, 1936).

Type observation. The sheet at PH (51684) is interpreted as the type of *Ainsliaea elegans* Hayata, based on "*Nakahara*, 3 Nov. 1905" on the label, but its locality is otherwise illegible.

Representative specimens examined. CHINA. **Yunnan:** "Inter pagum Dschungdien [Chungtien] et vieum Djitsung," *F. Handel-Mazzetti 7808*, Diar. Nr. 1429 (paratype of *Ainsliaea henryi* var. *subalpina* W, WU); Tsekou, *P. T. Wanberg 681* (paratype of *A. henryi* var. *ovatifolia*, K). TAIWAN. **Hsinchu:** The uppermost district of Takochin-chi, northern slope of Mt. Pintien, *T. Shimizu 20394* (S). **Ilan:** Mt. Nan-liu, Co. Ilan, *C. C. Chuang et M. T. Kao 2526*, p.p. (AAU); rd. from Nan-shan (Piyanan) to Mt. Chung-yang-chien, betw. Matakotou River & Pa, *M. Tamura et H. Koyama 23595* (S); Ilan Hsien, Taroko National Park, valley at ca. 3.5 km from Yunling Camp (ca. 12.5 km below Nanhutashan by trail), *W. L. Wagner 6515* (MO, US). **Nantou:** Mt. Neng-kao, betw. Yun-hai (Onoe) and Tien-chih (Noko), *M. Tamura et H. Koyama 23261* (S). **Nokosan:** Nokosan, Mt. Noko, betw. Noko Police Station & the Prefecture boundary, W of the divide, *H. H. Bartlett 6215* (NY, UC, US). **Taichung:** Mt. Hsueh-shan, between Shikayoutashan and the main peak, *M. Tamura 21079* (C). **Unknown:** Arisan, *R. Kanehira 2945* (NY); Mt. Morrison, *Kanehira 3051* (NY); Hassen-zan, *R. Kanehira 21194* (UC); Mt. Arisan, *Masumura in 1931* (MO); Mt. Taiheizan, *S. Suzuki in 1928* (MO); Mt. Taiheizan, *S. Suzuki im 1929* (UC); Mt. Chiagshi, Co. Hualien, *M. Teraya 459*, p.p. (CM); Mt. Morrison, 23 Aug. 1949, s. coll. (DAO).

16. Ainsliaea hypoleuca Diels ex H. Limpr., in Limpr., Feddes Repert. Beih. 12: 514. 1922. TYPE: China. Sichuan: Szetchuan, Kwan Hsien, 800–1000 m, 9 Apr. 1914, *H. Limpricht 1251* (lectotype, designated here, W!; duplicate, S!). Figure 29.

Ainsliaea petelotii Merr., J. Arnold Arbor. 21: 388. 1940. Syn. nov. TYPE: Vietnam. Tonkin: Cha Pa, massif du Song to Van, 1800 m, Apr. 1936, *A. Pételot 2082* (holotype, GH not seen; isotypes, MO!, NY!).

Plants perennial, herbaceous, 0.25–0.60 m tall; stems erect, unbranched, and leafy, lanuginose.

←

F = 1 mm; H, I = 0.5 mm; J, K = 2 mm; L = 1.5 mm; M, O = 2 mm; N, P = 1.5 mm. Drawn from A, D–L,l_2, *Tsang 28500* (US); B, *Henry 7461* (NY); C, *Wilson 2159* (W); L,l_1, *Li et al. 326* (CANB); M, N, the paratype *Handel-Mazzetti 7808* (W); O, P, the type *Handel-Mazzetti 8343* (W).

Figure 29. *Ainsliaea hypoleuca* Diels ex H. Limpr. —A. Habit. —B, C. Foliar trichomes. —D. Phyllaries. —E. Capitulum. —F. Chasmogamous floret. —G. Stamen of chasmogamous floret. -H. Style branches of chasmogamous floret. -I. Cleistogamous floret. Scale bars: A = 3 cm; B, C = 0.05 mm; D–G, I = 5 mm; H = 1 mm. Drawn from A, D–I, *Esquirol in 1917* (UC); B, C, *Bodinier & Ducloux in 1897* (US).

Leaves basally clustered, rosulate, long-petiolate; petioles broadly winged narrowing to the base, 2–7.5 × 0.7–1.4 cm (at the upper point); leaf blades chartaceous, ovate, 4–7.5 × 1.8–4 cm, apex acute to subobtuse, base rounded, margins obscurely denticulate; strongly discolorous, with upper surface deep green, sparsely strigose, and lower surface white strigose-lanate, with long crisped trichomes and long

straight trichomes, 2–3 mm; palmati-pinnate, 3-nerved; cauline leaves alternate, similar to the basal, gradually reduced upward to the base of the inflorescence. Capitula sessile or shortly pedunculate, arranged in a stachyoid inflorescence; involucre 6- or 7-seriate, cylindrical, 9–10 × 1.5–4 mm; phyllaries papyraceous, glabrescent to sparsely strigose above; outer phyllaries ovate, acute, ca. 1.5 × 0.8 mm; inner phyllaries linear to elliptic, acute to acuminate, midvein dark, ca. 8.5 × 1 mm. Florets 3, hermaphrodite, chasmogamous and cleistogamous (in single plant and in different seasons); corollas white (rarely pink); *chasmogamous florets* with corolla ligulate, ca. 9 mm, lobes ca. 4 mm (deeper slit ca. 5 mm), tube ca. 4 mm; anthers purple, ca. 7.5 mm, apical appendages truncate, ca. 1.5 mm, sagittate; tails ca. 1.8 mm, shortly pilose; style branches short, rounded, ca. 0.25 mm. Achenes oblong, ca. 2 mm, densely pilose, 10-nerved; pappus bristles 30 to 32, ca. 5 mm; *cleistogamous florets* with corolla tubulose to pseudoligulate, ca. 4 mm, 4- or 5-lobed, lobes 0.5 mm (deeper slit ca. 0.8 mm); anthers ca. 1.3 mm; style branches ca. 0.50 mm. Achenes 3.5–4 mm; pappus bristles ca. 7.5 mm.

Distribution and phenology. Valley with evergreen forest and in very dry open places in full sunlight or some shade, from southern and central China (Hubei, Sichuan, Yunnan) and northern Vietnam. Between 800 and 2700 m (Fig. 5A); collected in flower in January, February, April, and June.

Discussion. I propose to place *Ainsliaea petelotii* (which is characterized in the protologue by its leaves densely pubescent beneath and entirely glabrous above) into synonymy of *A. hypoleuca* because there is no diagnostic character that differentiates the former from the latter.

Because no herbarium was mentioned for the type specimen in the protologue of *Ainsliaea hypoleuca*, I propose the collection kept at W as lectotype because it presents the most complete plant.

Relationships. *Ainsliaea hypoleuca* resembles *A. latifolia* var. *latifolia*, sharing ovate leaves basally clustered, with the petioles broadly winged. *Ainsliaea hypoleuca* is distinguished by its strongly discolorous leaves, which are densely white-tomentose below but deep green and glabrous above.

Representative specimens examined. CHINA. **Hubei:** Shennongjia Forest District, vicinity of Houshanping on the S side of the Hou River, *B. Bartholomew et al. (Sino- Amer. Exped.) 1598* (CM, MO, UC). **Sichuan:** Tien-chuan-hsien, *K. L. Chu 2564* (W); "Kuang Hsien," *J. Esquirol in 1917* (UC); "Setschwan," in montium trans flumen Yalung ad septentr. oppidi Yenyüen, *F. Handel-Mazzetti 2615*, Diar. Nr. 491 (W); in montis Houdsengai prope oppidum Tetschang in valle Kientschang, *F. Handel-Mazzetti 1835*, Diar. Nr. 302 (W); "W-Setschwan, Wa-schan südl. v. Yatschou," *H. Weigold in 1915* (W); Muli Co., at the bank of Kata Jiang River, 24 Aug. 1983, *s. coll. 44* (HITBC). **Yunnan:** Environs Yunnan. *M. M. Bodinier & F. Ducloux in 1897* (US); Yunnan, *K. Hou 74248* (MO); NE Yunnan, Ma-li-ouan, *E. E. Maire s.n.* (W 7229, 7231); Siao-ho, *E. E. Maire 3155B* (UC); Yunnan. *E. E. Maire 3155* (NY); Yunnan-sen, Kunming, near Gingdian temple, *G. Murata 10486* (US); Malipo, *Q. W. Wang 83962* (HITBC). VIETNAM. Tonkin, *A. Pételot 1452* (NY); Tonkin, Cha Pa, *A. Pételot 4552* (paratype of *A. petelotii*, NY, US).

17. Ainsliaea latifolia (D. Don) Sch. Bip., Jahresber. Pollichia 18: 190. 1861. Basionym: *Liatris latifolia* D. Don, Prodr. Fl. Nepal: 169. 1825. TYPE: Nepal. Narainhetty "Perdicium ? triflorum," 24 Feb. 1803, *Hamilton s.n.* (holotype, BM not seen; isotype, K not seen, K photo LP!). Figure 30.

Ainsliaea pteropoda DC., Prodr. 7: 14. 1838. TYPE: India. "Ind. or.," *Royle s.n.* (lectotype, designated here, K!). Thailand. Chiang Mai: Western flank of Doi Inthanond, Mae Pau, 7 Dec. 1969, *C. F. v. Beusekon & C. Phengklai 2370* (epitype, designated here, BKF!; duplicates, AAU!, C!, CANB!).

Ainsliaea pteropoda DC. var. *silhetensis* DC., Prodr. 7: 14. 1838. *Ainsliaea silhetensis* (DC.) C. B. Clarke, J. Linn. Soc. 14: 411. 1875. TYPE: India. Silhet, *Wallich 2927, c.37* (lectotype, designated here, P not seen; duplicate, P photo LP!).

Ainsliaea heterantha Hand.-Mazz., Oesterr. Bot. Z. 87(1): 128. 1938. Syn. nov. TYPE: China. Yunnan: Yuen-kiang and Schaping (Schiping), Djutschin-Yako, 11 May 1935, *H. v. Wissmann 777* (holotype,W!).

Plants perennial, herbaceous, 0.25–0.70(1.20) m tall; stems erect, simple or few branched, with few leaves much reduced, loosely strigose. Leaves basally clustered, rosulate, long-petiolate; petioles broadly winged; leaf blades chartaceous, ovate, acute to subobtuse at the apex, base rounded, margins obscurely denticulate; palmati-pinnate, 3-nerved; cauline leaves alternate, short-petiolate to sessile, ovate to ovate-lanceolate. Capitula sessile or short-pedunculate, arranged in a stachyoid inflorescence; involucre 5- to 7-seriate, cylindrical, 8–10 × ca. 4 mm; phyllaries papyraceous; outer phyllaries ovate, acute, 2–3 × ca. 1 mm, apically strigose; inner phyllaries linear-oblong, acute, usually midvein dark, 7–12 × ca. 1 mm, glabrous or apically strigose. Florets 3, hermaphrodite, chasmogamous and cleistogamous (at different seasons or in single plant); corollas white, pink, or purplish; *chasmogamous florets* with corollas ligulate, 8–10 mm, lobes 2.5–4 mm (deeper slit 5–5.5 mm), tube 2.5–5 mm; anthers purplish, 4–5 mm, apical appendages rounded, ca. 0.6 mm, sagittate; tails 1–2 mm, shortly pilose; style branches short, truncate, ca. 0.5-0.7 mm. Achenes oblong, 1.8–2.5 mm, densely pilose, 10-nerved;

Figure 30. *Ainsliaea latifolia* (D. Don) Sch. Bip. A–J. *A. latifolia* var. *latifolia*. —A. Habit. —B–D. Foliar trichomes. —E. Phyllaries. —F. Cleistogamous floret. —G, H. Chasmogamous florets. —I. Style branches of chasmogamous floret. —J. Stamen of chasmogamous floret. —K. *A. latifolia* var. *taiwanensis* S. E. Freire. Scale bars: A, K = 3 cm; B, D = 0.05 mm; C = 0.1 mm; E = 3.5 mm; F–H = 2 mm; I, J = 1 mm. Drawn from A, E, G, I, J, *Drummond 25596* (UC); B–D, *Koyama et al. T-30524* (C); F, H, *Wissmann 777* (W); K, *Tamura & Koyama 23729* (S).

pappus bristles (sometimes absent) 29 to 34, 5–8 mm; *cleistogamous florets* with corollas tubulose to pseudoligulate, 2–5.5 mm, 5-lobed, lobes 0.25–0.75 mm (deeper slit 1–1.5 mm); anthers ca. 2 mm; style branches ca. 0.5 mm. Achenes 3.5–5.5 mm, densely pilose; pappus bristles 7–10 mm.

Distribution. Bangladesh, Bhutan, China (Guangdong, Yunnan), India, Indonesia (Sumatra), Myanmar, Nepal, Taiwan, and Thailand, at elevations between 1100 and 3600 m (Fig. 5B).

Discussion. The protologue of *Ainsliaea pteropoda* includes the specimen *Wallich 2927, c.37* from Nepal and the collection *Royle s.n.* from "Ind. or." The first was located at W, and the second at K. I propose the material "Ind. or., *Royle s.n.*" kept at K, which presents the most complete plant, as lectotype of *A. pteropoda*. However, because the leaves of the specimen *Royle s.n.* are not well preserved, I also propose the collection *C. F. v. Beusekon & C. Phengklai 2370* kept at BKF, which is a widely distributed collection (duplicates seen at AAU, C, CANB) and agrees with the protologue of *A. pteropoda*, as epitype.

According to the protologue, *Ainsliaea pteropoda* DC. var. *silhetensis* DC., was based on specimen *Wallich cat. 2927, c.37* from Silhet. I was unable to locate this collection. However, in P, I found a sheet from Schulz Bip.'s herbarium with the label "C-37(Wallich)" (on the same sheet there is another plant from Jackemont's herbarium n. 490). Even if the label of the specimen "C-37(Wallich)" does not show the locality mentioned in the protologue (i.e., Silhet), the sheet has Schulz Bip.'s annotations on the right-hand side ("... involucro opaco, subvilloso"), which are in accordance with the protologue of *A. pteropoda* var. *silhetensis*, i.e., "...invol. parce lanuginosis" (vs. "involucra glabrata" in *A. pteropoda* DC. from Nepal). I therefore selected the specimen *Wallich 2927, c.37* at P as lectotype of *A. pteropoda* var. *silhetensis*.

Ainsliaea pteropoda var. *silhetensis* was said to differ from *A. pteropoda* by its involucral bracts lanuginose. However, subsequent collections have shown this character to be within the range of variation for the species.

Handel-Mazzetti (1938) described *Ainsliaea heterantha* on the basis of its chasmogamous and cleistogamous florets. However, this is a variable feature throughout the range of *A. latifolia*. Therefore, the possession of chasmogamous and cleistogamous florets rules out the possibility of separate taxonomic recognition of *A. heterantha*, and this otherwise similar species is synonymized here.

Relationships. *Ainsliaea latifolia* most resembles *A. bonatii*, *A. henryi*, *A. hypoleuca*, *A. macrocephala*, *A. qianiana*, and *A. spicata*, those species with winged petioles. *Ainsliaea henryi* is distinguished by its achenes glabrous or apically pilose (vs. these other species with achenes consistently pilose). Five taxa, *A. bonatii*, *A. hypoleuca*, *A. latifolia*, *A. qianiana*, and *A. spicata*, may be differentiated by their leaf blades, i.e., ovate and rounded at the base in *A. latifolia*. *Ainsliaea bonatii* has cordate leaf blades, whereas *A. spicata* has obovate or obovate to pandurate leaf blades. *Ainsliaea qianiana* is distinguished by its subglabrous and somewhat fleshy leaves. In contrast, *A. hypoleuca* has discolorous leaves that are white-tomentose below. *Ainsliaea macrocephala* may be distinguished by its larger involucre, 13–15 mm, as well as by its foliar trichomes T-shaped.

Ainsliaea latifolia is recognized in this treatment with two varieties, their distributions occasionally overlapping in Thailand. Both varieties form a reticulate pattern of morphologically intergrading populations.

Key to the varieties of Ainsliaea latifolia *in Southeastern Asia*

1a. Leaf blade ovate, petiole commonly equal, rarely longer than the blade. Leaves strigose (with long straight trichomes only) or strigose-lanate below (with long straight trichomes and long crisped trichomes) . . . . . 17a. var. *latifolia*

1b. Leaf blade elliptic or narrowly ovate, petiole equal to or more commonly longer than the blade. Leaf subglabrous (with few straight trichomes only) . . . . . 17b. var. *taiwanensis*

17a. Ainsliaea latifolia (D. Don) Sch. Bip. var. **latifolia.** Figure 30A–J.

Leaves long-petiolate, petioles (2)4–9(11) × 4–30 mm (at the upper point); leaf blade ovate, (2)5–10 × (1.5)3–8 cm, apex acute to subobtuse; concolorous, strigose on both surfaces, with long straight trichomes or more commonly slightly discolorous, with upper surface sparsely strigose, and lower surface white strigose-lanate, with a combination of the following trichomes types: short glandular biseriate trichomes; long crisped trichomes; long straight trichomes, 0.3–0.8 mm. Capitula solitary or 2- to 5-fasciculate, subtended by reduced leaves or leaf-like bracts. Florets usually chasmogamous (sometimes without pappus), more rarely cleistogamous.

Distribution and phenology. Bangladesh, Bhutan, China (Guangdong, Yunnan), India, Myanmar, Nepal, and Thailand, collected from evergreen forest, usually in sunny places, among rock, from elevations between 1100 and 3600 m; flowering and fruiting throughout the year.

Representative specimens examined. BANGLADESH. "East Bengal," *Griffith 3331* (S); "East Himalaya," *Griffith 3331* (W). BHUTAN. "Bootan." no further data (W 7214.

7215). CHINA. **Guangdong:** Hong Kong, Hyszen, *Exped. Biolog. Sino-Rossica 925* (NY). **Yunnan:** Central Yunnan, Kunming, *P. I. Chiou 59608* (CM); "Inter fluvios Djinschadjang ("Yangtse") et Landsang-djiang (Mekong)," *F. Handel-Mazzetti 8501*, Diar. Nr. 1588 (W); "Inter pagos Yungning et Dschungdien [Chungtien], in regionis calide temperatae primetis supra vicum Tschwadse," *F. Handel-Mazzetti 7623*, Diar. Nr. 1380 (W); "Szemao," *A. Henry 12763* (NY, US); "Szemao." *A. Henry 12.763B* (MO); NE Yunnan, So?-kou, Sep. s.d., *E. E. Maire s.n.* (W 7227); s. loc., *R. Mauri in 1921* (UC); Xujiaba, Jingdong Co., 28 Apr. 1981, s. coll. (HITBC 071880); Bijiang Co., behind the hill of the Middle School, 26 May 1976, s. coll. (HITBC 076243); Bijiang Co., Pihe logging center, 20 June 1978, s. coll. (HITBC 076246); Bijiang Co., Nabajiao, 8 July 1978, s. coll. (HITBC 076247); Qiubei Co., 28 Apr. 1962, s. coll. (HITBC 076250); "Zhengyuan" [Zhenyuan] Co., Sep. 1974, s. coll. (HITBC 076252); Ageju, Fugong Co., 9 Oct. 1987, s. coll. (HITBC 076258, 076265); Songming Co., 5 Sep. 1965, s. coll. (HITBC 076262). INDIA. **Assam:** Khasia, Reg. Temp., *J. D. Hooker & T. Thomson s.n.*, p.p. (C, M, NY, S, UC, W); Assam, above Karong, Manipur, *W. Koelz 27006* (UC); Eastern Circle, Shillong, Kameng F.D. (N.E.F.A.), Bompu up hill next to Cauteen, *G. Panigrahi 6184* (CAL); Naga Hills, Kohima?, *D. Prain 23896* (M); Khasia Hills, *Simond 30405* (M). **Himachal Pradesh:** Himalaya Mtns., above Gangotry, *W. Dudgeon & L. A. Kenoyer 36* (MO, PH); Simla, *R. Singh 12786* (M). **Manipur:** Ukhrul, *F. Kingdon Ward 17086* (NY); Mao, "11 Feb. 54," *s. coll. 1761* (CAL). **Punjab:** Kasauli, *J. R. Drummond 20055* (UC); Punjab, *J. R. Drummond 25596* (UC). **Sikkim:** Reg. Temp., *J. D. Hooker & T. Thomson s.n.* (C, M, S, W); Sikkim, *J. D. Hooker & T. Thomson s.n.* (W 16496); Darjeeling, *W. Koelz 10517* (M); "Darshiling," *O. Kuntze 6695* (NY); Sikkim, *A. Meebold 838* (S). **Uttar Pradesh:** NW India, Mussoorie, *R. Kirat in 1932* (NY, S), *R. Kirat in 1926* (S); Mussoorie, *R. R. Stewart 15787* (NY). **Unknown:** Joacinsan?, Bemo Kathijan?, *J. F. Duthie 12985* (CAL); no further data, *Jackemont 490* (P); NW Himalaya, s.d., *Mackinnon s.n.* (CAL 253998A); NW Himalaya, *Stolitzka in 1864* (W); "Ind. Orient.," no further data, *Wallich C-44* (S). MYANMAR [BURMA]. North Burma, Hpare Pass, *F. Kingdon Ward 447* (NY); Burma, Sirhoi, *F. Kingdon Ward 18260* (NY); NE Burma, Kambaiti, 73 km E of Myitkyina, *R. Malaise 158* (S), *R. Malaise 186* (S). NEPAL. Chusiakana, *Biskam 13* (UC); Kali Gandaki, above Ulleri, *Clifford G. Rice 10-78* (US); Nepalia, "Kamaon Ind. or.," *J. D. Hooker & T. Thomson s.n.* (NY, W); Bagmati zone, NW slope of Nagarjung Hill, *D. H. Nicolson 2955* (US); "Nepalia, 1821," *Wallich s.n.* (M, NY, P (photo LP), W). THAILAND. **Chiang Mai:** Doi Inthanond, *K. Bunchuai 1437* (BKF, C); Doi Chiang Dao, *R. Geesink et al. 8170* (AAU, BKF, C); Doi Inthanon, *H. Koyama & C. Phengklai T-39997* (BKF); Doi Pha Hom Pok, Fang Distr., *H. Koyama et al. T-33444* (BKF); Doi Inthanond, *T. Smitinand & I. Alsterlund 6695* (BKF); Chiangmai, Doi Chiengdao, *T. Smitinand & J. A. R. Anderson 7323* (BKF); Inthanond, *T. Smitinand et al. 10299* (BKF); Chiangmai, "Doi Angka," *Winit 1344* (BKF). **Kanchanaburi:** Khao Kamphaeng, Tham Than Lod National Park, Srisawat Distr., *H. Koyama et al. T-30524* (AAU, BKF, C). **Unknown:** "Siam Forest Service, Doi Dawk, Charomtawng," *H. Garrett 618* (AAU, BKF).

17b. Ainsliaea latifolia (D. Don) Sch. Bip. var. **taiwanensis** S. E. Freire, var. nov. TYPE: Taiwan, Ilan Hsien, Taroko Nat. Park, valley at 3.5 km from Yunling Camp (ca. 12.5 km below Nanhutashan by trail), 7 Sep. 1991, *W. L. Wagner 6516* (holotype, MO!). Figure 30K.

Leaves very long-petiolate with petioles broadly winged, 5–11 × 6–18 mm (at the upper point); leaf blades elliptic or narrowly ovate, (2.5)4.5–9 × 1.5–3(3.5) cm, apex acute; subglabrous on both surfaces with long straight trichomes along the main veins. Capitula solitary or 2- to 5-fasciculate. Florets usually cleistogamous.

Distribution and phenology. Collected from mossy sites in evergreen forest by streams, between 1700 and 2650 m, from Taiwan, Indonesia (Sumatra), and southern Thailand; flowering in January, February, May, and August, flower bud in June, July, September, October, and December.

Discussion. Most collections of var. *latifolia* have pilose leaves, with ovate leaf blades. However, collections principally from Taiwan (also a few collections from Thailand and only one from Sumatra) are nearly glabrous, with narrowly ovate leaf blades and petiole equal to or more commonly longer than the blade. Otherwise they resemble var. *latifolia*. Consequently, I propose the new variety var. *taiwanensis*.

Representative specimens examined. INDONESIA. **Sumatra:** Sidikalang, *H. Koerper s.n.* (M). TAIWAN. **Ilan:** Szu-yuan, Ilan Co., *C. C. Chuang & M. T. Kao 2474* (AAU, UC); Ilan Co., Taipingshan, *S. F. Huang 4990* (CANB, CM); Ilan, rd. from Mt. Chung-yang-chien to Nan-shan (Piyanan), along Hagapalis River, *M. Tamura & H. Koyama 23729* (S); Ilan, rd. from Mt. Chung-yang-chien to Nan-shan (Piyanan), betw. Matakotou River & Kurimukan, *M. Tamura & H. Koyama 23780* (S). **Nan-tou:** Nan-tou, Mt. Neng-kao, betw. Tien-chin (Noko) & Yun-hai (Onoe), *M. Tamura & H. Koyama 23388* (S). **Unknown:** Mt. Taiheizan, *S. Suzuki 549* (S), *S. Suzuki 9513* (S). THAILAND. **Chiang-Mai:** Doi Anga-Kette, *C. C. Hosseus 358a* (MO, C, M, W); Doi Pha Hom Pok, Fang Distr., *H. Koyama et al. T-33435* (AAU, BKF, C); Doi Inthanod, *G. Murata et al. T-15998* (BKF, C); Doi Inthanod, Payap, *M. Tagawa et al. T-2866* (BKF). **Unknown:** Doi Anga, *H. Garrett 643* (BKF).

18. Ainsliaea macrocephala (Mattf.) Y. C. Tseng, Acta Phytotax. Sin. 31(4): 364. 1993. Basionym: *Ainsliaea pteropoda* var. *macrocephala* Mattf., Notizbl. Bot. Gart. Berlin-Dahlem 11: 107. 1931. TYPE: China. Yunnan: Likiang, 3500 m, 1 Sep. 1914, *C. Schneider 2328* (lectotype, designated here, K!; duplicates, K photo LP!, US!). Figure 31.

Plants perennial, herbaceous, 0.45–0.60 m tall; stems erect, unbranched, with few leaves much reduced, lanuginose. Leaves basally clustered, rosulate, long-petiolate with petioles broadly winged, slightly narrowed to the base, 5–8 × 0.7–0.9 cm (at the upper point); leaf blades slightly fleshy, ovate,

Figure 31. *Ainsliaea macrocephala* (Mattf.) Y. C. Tseng. —A. Habit. —B. Foliar trichome. —C. Phyllaries. —D. Capitulum. —E. Chasmogamous floret. —F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 0.3 mm; C–E = 5 mm; F = 2 mm; G = 1 mm. Drawn from the paratype *Rock 5773* (US).

2.5–7 × 1.5–4.5 cm, apex acute to subobtuse, base rounded, margins obscurely denticulate; sericeous on both surfaces with long T-shaped trichomes, 3–4 mm; palmati-pinnate, 3-nerved; cauline leaves alternate, short-petiolate to sessile, ovate to lanceolate, ca. 3 × 1.5 cm. Capitula sessile or short-pedunculate, arranged in a stachyoid inflorescence; involucre 6- or 7-seriate, cylindrical, 13–15 × 2.5–4 mm; phyllaries

subcoriaceous, margins purple, densely strigose; outer phyllaries ovate, acute, 3–3.5 × 2 mm: inner phyllaries linear, acuminate, midvein dark, 9–14 × 0.7–1 mm. Florets 3, hermaphrodite, chasmogamous: corollas deep rose, ligulate, 13–14 mm, lobes 5–7 mm (deeper slit 7–8 mm), tube 5–7 mm; anthers dark red, 7–7.4 mm, apical appendages rounded, 1–1.5 mm. sagittate; tails smooth to slightly pilose, 2.2–3 mm; style branches short, rounded, 0.3–0.5 mm. Achenes oblong, ca. 4 mm, 11-nerved, densely pilose; pappus bristles ca. 55, 7.5–9 mm.

Distribution and phenology. Collected from alpine conifer forest in China (Yunnan), from dry stony sites among scrub, from elevations between 3500 and 3600 m; flowering in August and September.

Discussion. Because no herbarium was mentioned for the type specimen in the protologue of *Ainsliaea pteropoda* var. *macrocephala*, I propose the collection kept at K as lectotype because it is better preserved.

Relationships. *Ainsliaea macrocephala* most resembles *A. latifolia*. Both have ovate leaves that are long-petiolate, with winged petioles. They may be differentiated by their leaf trichomes and involucres, i.e., *A. macrocephala* has larger involucres, 13–15 × 2.5–4 mm and foliar trichomes T-shaped (vs. 8–10 × ca. 4 mm and a combination of long straight and long crisped trichomes, respectively, in *A. latifolia*).

Representative specimens examined. CHINA. **Yunnan:** Likiang valley, *G. Forrest 6545* (paratype, UC, W); Prope urbem Lidjiang ("Likiang") in Mt. Yülung-schan, *F. Handel Mazzetti 3619* (W); Likiang, *J. F. Rock 5628* (paratype, NY), *J. F. Rock 5773* (paratype, UC, US); Zhongdian Co., Biandong, 19 June 1963, s. coll. (HITBC 076263).

19. Ainsliaea mairei H. Lév., in Le Monde des Plantes 18: 31. 1916. TYPE: China. Yunnan: Mt. Io-Chan, 3200 m, Nov. 1912, *E. E. Maire s.n.* (holotype, E!). Figure 32.

Plants perennial, herbaceous, ca. 0.40 m tall; stems erect, unbranched, leafy, strigose. Leaves basally clustered, rosulate, sessile; leaf blades chartaceous, linear to obovate, 8–14 × 1.5–2.5(3.5) cm, apex acute, attenuate at the base, margins entire to obscure denticulate; slightly discolorous, upper surface dark green, under surface paler, sericeous on both surfaces with long T-shaped trichomes, ca. 2.5–3 mm; palmati-pinnate, 3-nerved; cauline leaves alternate, sessile, linear to elliptic, gradually reduced upward to the base of the inflorescence, 2.5–4 × 0.5–0.8 cm. Capitula sessile, arranged in a stachyoid inflorescence; involucre 5- to 7-seriate, narrowly campanulate, 10–12 × 4–7 mm; phyllaries subcoriaceous, purple; outer phyllaries ovate, acute, ca. 3 × 1 mm, dorsally strigose; inner phyllaries linear to elliptic, acute, midvein dark, ca. 12 × 2 mm, glabrous. Florets, 2 or 3, hermaphrodite, chasmogamous; corollas pink, ligulate, 10–12 mm, lobes ca. 5–6 mm (deeper slit 6–7 mm), tube 4–5 mm; anthers deep rose, 4.5–7 mm, apical appendages rounded, ca. 1 mm, sagittate; tails 1.5–2 mm, smooth to slightly pilose; style branches short, rounded, ca. 0.4 mm. Achenes oblong-obovate, ca. 4 mm, 10-nerved, densely pilose, pappus bristles ca. 36, ca. 9 mm.

Distribution and phenology. China (Sichuan, Yunnan), growing on mountain slopes between 1800 and 3500 m, commonly on sandy soils; collected in flower in September and November.

Relationships. *Ainsliaea mairei* resembles *A. macrocephala* and *A. yunnanensis*, species with villose leaves (T-shaped trichomes) and capitula arranged in unilateral spikes. *Ainsliaea mairei* may be distinguished by its leaves linear to obovate, slightly narrowed at the base (vs. ovate and abruptly contracted at the base in *A. macrocephala* and *A. yunnanensis*).

Representative specimens examined. CHINA. **Sichuan:** Prope castellum Kwapi ad septentr. oppidi Yenyüen, *F. Handel-Mazzetti 2485*, Diar. Nr. 977 (W). **Yunnan:** "Yünnanfu" [Kunming], *J. Cavalerie 20* (S); "Yünnanfu," Mt. Landjigu, *F. Handel-Mazzetti 5683*, Diar. Nr. 991 (W); "Yun-nan-sen," *E. E. Maire 921* (UC); "Tong-Tchouan," *E. E. Maire 3936* (NY, S, UC); "Yünnanfu-Suifu," *R. Mell in 1914* (W).

20. Ainsliaea nana Y. C. Tseng, Acta Phytotax. Sin. 31 (4): 365. 1993. TYPE: China. Sichuan: Tianquan, 27 Aug. 1948, *Chung-luen Wu 12307* (holotype, NAS not seen).

Plants perennial, herbaceous, 0.11–0.18 m tall; stems erect, unbranched, naked. Leaves basally clustered, rosulate, long-petiolate, petioles wingless, 1–2.3 cm; leaf blades chartaceous, ovate, 7–16 × 5–10 mm, obtuse at the apex, base slightly cordate, margins denticulate; upper surface green, lower surface purple; palmati-pinnate, 3-nerved. Capitula pedunculate, arranged in thyrsoid inflorescence; peduncles 5–15 mm long, glabrous; involucre 5-seriate, cylindrical, ca. 3 mm broad; phyllaries purple, glabrous; outer phyllaries ovate or ovate to lanceolate, obtuse, 1–3.5 × 0.8–1 mm; inner phyllaries lanceolate to linear or oblong, acute to acuminate, ca. 7 × 0.7–0.9 mm. Florets 3, hermaphrodite, chasmogamous; corolla ligulate, ca. 9 mm, lobes 4–5 mm; anthers ca. 7 mm, apical appendages rounded, sagittate; tails ca. 1 mm; style branches short, rounded, ca. 1 mm. Achenes obovate, ca. 4 mm, 10-nerved, glabrous; pappus bristles ca. 4 mm, margins plumose.

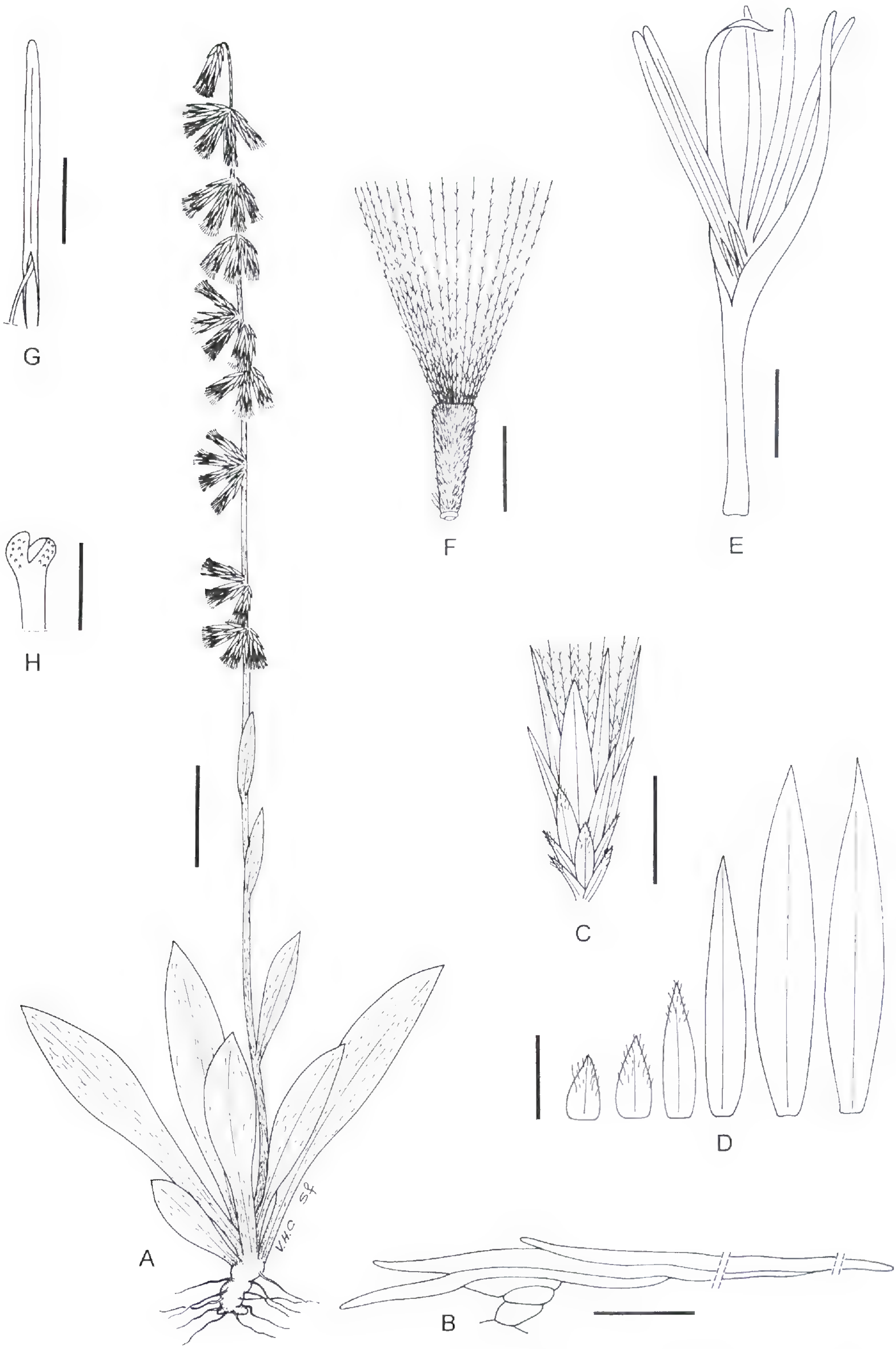


Figure 32. *Ainsliaea mairei* H. Lév. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Phyllaries. —E. Chasmogamous floret without pappus and ovary. —F. Achene with pappus. —G. Stamen. —H. Style branches. Scale bars: A = 3 cm; B = 0.2 mm; C = 5 mm; D, F = 3 mm; E, G = 2 mm; H = 1 mm. Drawn from A, C–H, *Maire 3936* (S); B, the holotype *Maire in 1912* (E).

Distribution and phenology. At present known only from the type collection from Sichuan (China) at 2400 m; flowering in August.

Relationships. *Ainsliaea nana* seems to be distinguished by its small habit, leaves long-petiolate, with leaf blades ovate-cordate and purplish below, and achenes glabrous.

Unfortunately, no material was available from this species, so the description is based on the original diagnosis.

Representative specimens examined. CHINA. **Sichuan:** Mt. Emei, *Guanghui Yang 57464, 57474* (paratype, NAS not seen), *Xian-yu He 6356* (paratype, NAS not seen); Anxian, *Wen-Kwang Hu 13377* (paratype, NAS not seen).

21. Ainsliaea nervosa Franch., Bull. Mus. Hist. Nat. (Paris) 1: 64. 1895. TYPE: China. Yunnan: Long-Ki, Apr. 1894, *J. M. Delavay 4939* (holotype, P not seen; isotypes, P photo LP!, P!). Figure 33.

Plants perennial, herbaceous, 0.25–0.50 m tall; stems erect, unbranched, leafy, subglabrous. Leaves basally clustered, rosulate, long-petiolate with petioles wingless, petioles 6–10 cm long, flat; leaf blades subcoriaceous, narrowly elliptic to narrowly lanceolate, 9–10 × 2–2.5 cm, apex acute, base longly attenuate into the petiole, margins obscurely denticulate; upper surface glabrous, deep green, and lower surface paler, loosely strigose, paler, glabrate at maturity, with long straight trichomes; palmati-pinnate, 3-nerved, these major veins subparallel; cauline leaves alternate, sessile, linear to elliptic, much reduced. Capitula short-pedunculate, arranged in thyrsoid inflorescence; involucre 5- or 6-seriate, broadly cylindrical, ca. 10 × 5–7 mm; phyllaries papyraceous, all with long biseriate glandular trichomes; outer phyllaries ovate, acute, ca. 2 × 1.3 mm; inner phyllaries linear to elliptic, acute, midvein dark, ca. 10 × 1 mm. Florets 3, hermaphrodite, chasmogamous and cleistogamous (at different or same seasons); corollas pink; *chasmogamous florets* with corollas ligulate, ca. 8.5 mm, lobes ca. 4 mm (deeper slit ca. 4.5 mm), tube ca. 4 mm; anthers ca. 3.5 mm, apical appendages rounded, ca. 0.7 mm, sagittate; tails ca. 1 mm, shortly pilose; style branches short, rounded, ca. 0.3 mm. Achenes obovate to elliptic, ca. 2 mm, 10-nerved, densely pilose; pappus bristles 25 to 30, ca. 6 mm, white; *cleistogamous florets* with corollas tubulose to pseudoligulate, ca. 4.2 mm, lobes ca. 0.3 mm (deeper slit ca. 0.5 mm); anthers ca. 2 mm; style branches ca. 0.4 mm. Achenes ca. 2.5 mm; pappus bristles ca. 6 mm.

Distribution and phenology. China (Sichuan, Yunnan), collected on rocky slopes along the stream at elevations between 1500 and 1800 m; flowering in April, fruiting in October and November.

Relationships. *Ainsliaea nervosa* is closely related to *A. glabra* var. *sutchuenensis*. Both taxa have leaves long-petiolate, achenes pilose, and corollas pink. However, the leaves in *A. nervosa* are narrowly lanceolate, and the phyllaries are papyraceous with long biseriate glandular trichomes. On the contrary, *A. glabra* var. *sutchuenensis* has leaves broadly elliptic and phyllaries that are glabrous or scarcely strigose, with short biseriate glandular trichomes.

Representative specimens examined. CHINA. **Sichuan:** O-Pien Hsien, *Y. S. Liu 2325* (US); Ping-shan Hsien, *F. T. Wang 22682* (KUN); Mt. Omei, *E. Faber 128* (K, in the same sheet as the type of *A. glabra*, i.e., *E. Faber 391*).

22. Ainsliaea paucicapitata Hayata, Icon. Pl. Formos. 8: 71. 1919. TYPE: Taiwan. Nökösan, 9000 ft., Jan. 1908, *U. Mori s.n.* (type, TI not seen; TI photo LP!). Figure 34.

Plants perennial, herbaceous, ca. 0.20 m tall; stems erect, unbranched, naked. Leaves basally clustered, rosulate, long-petiolate with petioles wingless, 1–1.5 cm; leaf blades deltoid, 1–1.5 × 1–1.5 cm, acute at the apex, base cordate, margins obscurely denticulate; palmati-pinnate, 3- to 5-nerved. Capitula sessile, arranged in a stachyoid inflorescence; involucre campanulate, ca. 10 mm; outer phyllaries ovate, inner phyllaries linear to lanceolate. Florets 3, hermaphrodite, chasmogamous; corollas ligulate, ca. 10 mm, 5-lobed, lobes ca. 5 mm. Achenes oblong to elliptic, densely pilose; pappus bristles with margins plumose.

Distribution and phenology. This species, at present, is only known from type locality of Taiwan (Nökösan) at ca. 3000 m; flowering in January.

Relationships. *Ainsliaea paucicapitata* resembles *A. apiculata* var. *apiculata*, from Japan. Both species have leaves long-petiolate, with leaf blades that are subrotundate, and the capitula arranged in spikes. *Ainsliaea paucicapitata* is cleary distinguished by its leaf blades that are shallowly 5-lobed (vs. shallowly 5-lobed to 5-angled) and its leaves basally rosulate (vs. leaves clustered above stem base).

Unfortunately, no material was available from this species, so the description is based on the original diagnosis by Hayata (1919) and the photo of the type distributed by TI.

23. Ainsliaea pentaflora S. E. Freire, Novon 12: 451. 2002. TYPE: Vietnam. Tonkin: Massif du

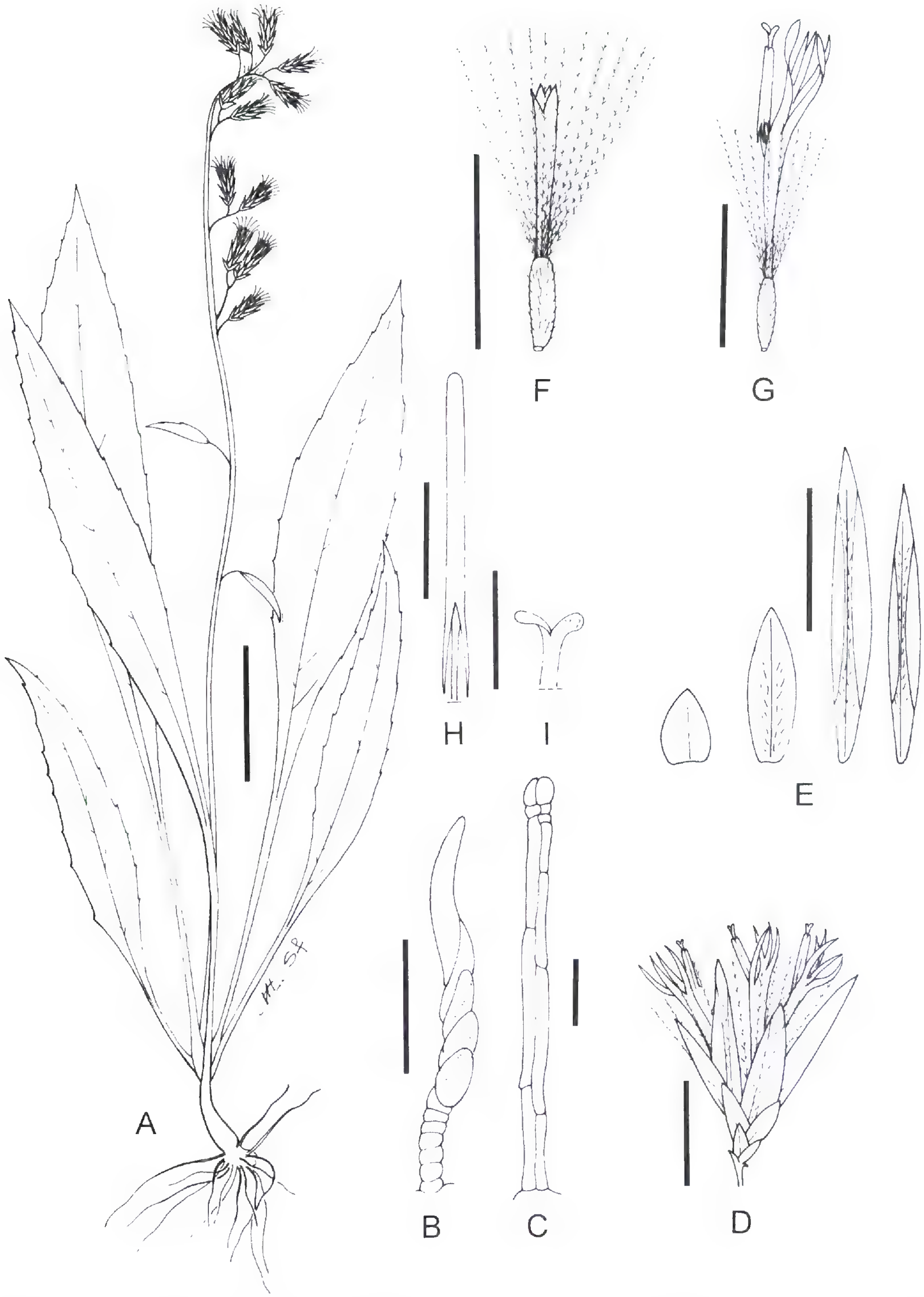


Figure 33. *Ainsliaea nervosa* Franch. —A. Habit. —B, C. Foliar trichomes. —D. Capitulum. —E. Phyllaries. —F. Cleistogamous floret. —G. Chasmogamous floret. —H. Stamen of chasmogamous floret. —I. Style branches of chasmogamous floret. Scale bars: A = 3 cm; B, C = 0.1 mm; D–G = 5 mm; H, I = 1.5 mm. Drawn from A–E, G–I, the isotype *Delavay 4939* (P); F, *Liu 2325* (US).

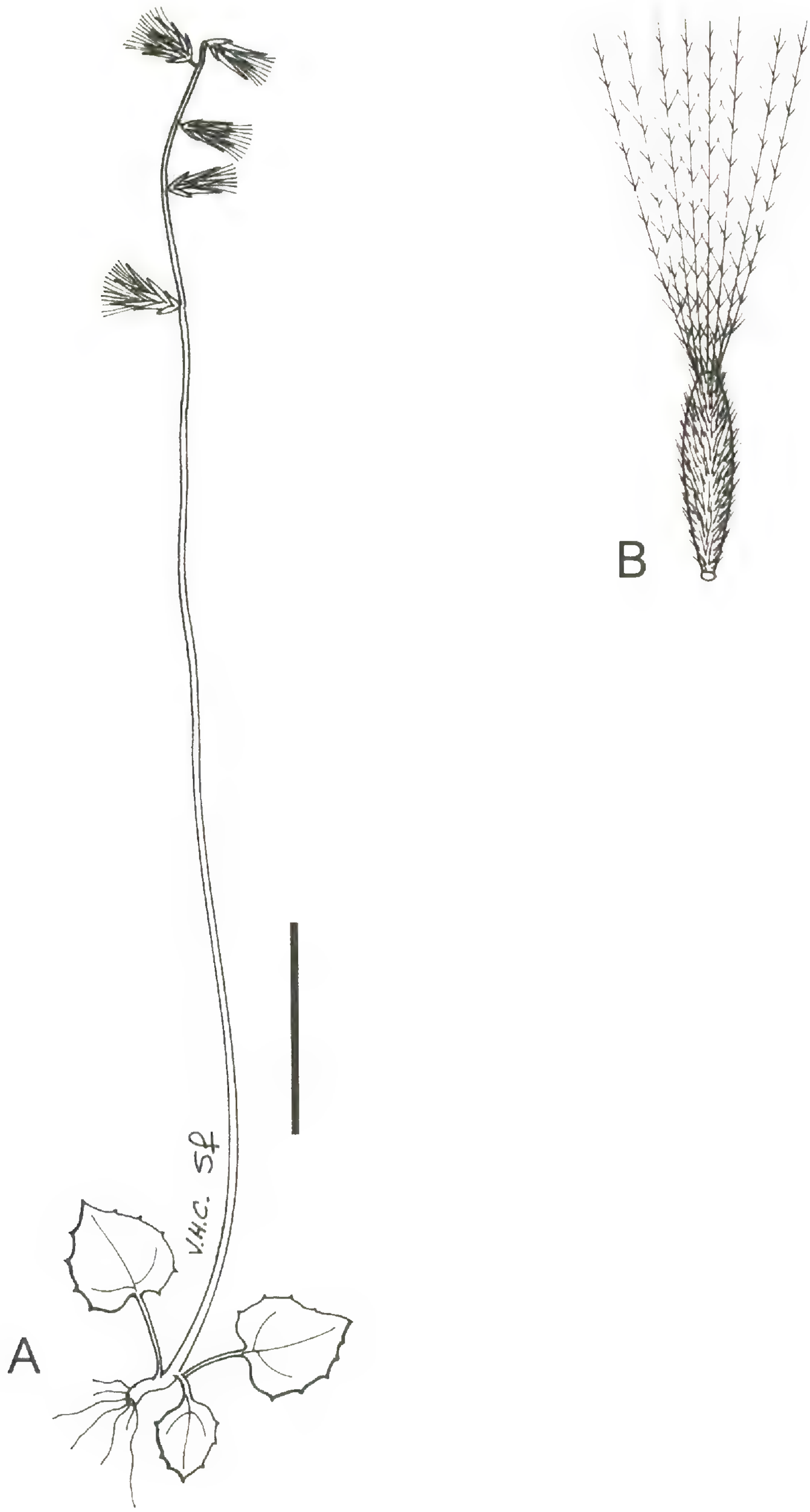


Figure 34. *Ainsliaea paucicapitata* Hayata. —A. Habit. —B. Achene with pappus. Scale bars: A = 3 cm; B = (scale unknown). Drawn from A, the photo of type *U. Mori s.n.* (TI); B, redrawn from an outline of the photo of the type.

Tom Dav, 900 m, Dec. 1921, *A. Pételot 417* (holotype, C!; isotype, NY!).

Plants perennial, herbaceous, 0.35–0.45 m tall; stems erect, unbranched, leafy, strigose. Leaves basally clustered, rosulate, long-petiolate; petioles wingless, 6.5–9 cm long, strigose; leaf blades chartaceous, ovate, 7.5–11 × 3.5–6 cm, apex acute, base abruptly rounded, then cuneately narrowed into the petiole, margins denticulate; upper surface slightly scabrous, sparsely strigose to glabrous, with straight trichomes, 0.7–1.25 mm, lower surface moderately strigose, veins somewhat more densely pubescent; palmati-pinnate, 3-nerved; cauline leaves few, much reduced, ovate to elliptic. Capitula sessile, arranged in thyrsoid inflorescences; involucre 6- or 7-seriate, narrowly campanulate, 13–15 × 12–14 mm; phyllaries coriaceous, apically villose; outer phyllaries oblong to ovate, acute, ca. 4 × 1.5 mm; inner phyllaries linear to elliptic, acute, midvein dark, 10–10.5 × 1.5–1.7 mm. Florets 5, hermaphrodite, cleistogamous; corollas tubulose to pseudoligulate, 4.5–5.5 mm, 5-lobed, lobes ca. 1 mm (deeper slit ca. 1.5 mm); anthers ca. 3 mm, apical appendages truncate, ca. 0.4 mm, sagittate; tails 0.8 mm, shortly pilose; style branches short, obtuse, ca. 1.2 mm. Achenes oblong, ca. 4 mm, 10-nerved, pilose; pappus bristles ca. 39, 7.5–8 mm.

Distribution and phenology. Vietnam (Tonkin), between 900 and 1400 m (Fig. 3B); collected in flower in April and December.

Relationships. *Ainsliaea pentaflora* most resembles *A. latifolia*. Both have ovate leaves arranged in a basal rosette and apical anther appendages that are rounded. *Ainsliaea pentaflora* differs from *A. latifolia* by its 5-flowered capitula, and its practically wingless petioles. In contrast, the capitula of *A. latifolia* are 3-flowered and the petioles are winged. Two other species of the genus also present capitula 5-flowered, i.e., *A. brandisiana* from Burma and *A. spanocephala* Y. C. Tseng from Thailand. Both species have acuminate apical anther appendages (vs. truncate apical anther appendages in *A. pentaflora*).

Representative specimens examined. VIETNAM. **Tonkin:** Massif du Tom Dav, *A. Pételot 4551* (NY, US).

24. Ainsliaea pertyoides Franch., J. Bot. (Morot) 2: 70, t. 3. 1888. TYPE: China. Yunnan: In dumetis, solo calcareo, ad fauces Kien-min-keou, prope Mo-so-yn, 23 Jan. 1885, *J. M. Delavay 1218* (lectotype, designated here, P!). Figure 35.

Ainsliaea sparsiflora Vaniot, Bull. Acad. Int. Géogr. Bot. 12: 118. 1903. *Ainsliaea pertyoides* f. *sparsiflora* (Vaniot) Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 384. 1909. *Ainsliaea pertyoides* var. *sparsiflora* (Vaniot) H. Lév., Cat. pl. Yun-Nan: 37. 1915. TYPE: China. Yunnan: "Environs de Yu-nan-sen, bois de la pagode de He-long-tan," 24 Feb. 1897, *E. Bodinier 79* (holotype, P!; isotype, E!).

Small shrubs, 0.50–0.80 m tall; stems erect, branched and leafy above, with numerous axillary branches, simple and naked below the inflorescence, strigose. Leaves distichous, alternate, short-petiolate with petioles wingless, 2–3 mm; leaf blades subcoriaceous, ovate to oblong, 4–5.5 × 2–3 cm, apex acuminate, rounded to slightly cordate at the base, margins entire to obscurely denticulate; palmati-pinnate, 3-nerved. Branch leaves smaller than the others of the principal stem. Capitula sessile or short-pedunculate, arranged in thyrsoid and leafy inflorescence; involucre 5- or 6-seriate, cylindrical to campanulate, ca. 14 × 5 mm; phyllaries subcoriaceous, apically strigose; outer phyllaries ovate or ovate to elliptic, acute, 1–3 × 1–1.5 mm; inner phyllaries linear to elliptic, acute, midvein dark, ca. 13 × 1.5 mm. Florets 3, hermaphrodite, chasmogamous; corolla white, pink above, ligulate, ca. 13 mm, lobes ca. 5 mm (deeper slit ca. 6 mm), tube ca. 7 mm; anthers ca. 7.5 mm, apical appendages truncate, ca. 1 mm, sagittate; tails ca. 2 mm, shortly pilose; style branches short, rounded, ca. 0.5 mm. Achenes oblong, ca. 3 mm, 10-nerved, densely pilose; pappus bristles ca. 40, ca. 7.5 mm.

Relationships. *Ainsliaea pertyoides* appears to occupy an isolated possition in the genus by the distinctive stem branched and leafy above. This habit resembles the genus *Pertya*. However, the pappus bristles with margins plumose of *A. pertyoides* (vs. scabrid in *Pertya*) relate this species to *Ainsliaea*.

Distribution. Restricted to southwestern China (Guizhou, Yunnan), between 1500 and 2600 m (Fig. 6B).

Discussion. Three specimens from Yunnan are mentioned in the protologue of *Ainsliaea pertyoides*: "In dumetis, solo calcareo, ad fauces Kien-min-keou, prope Mo-so-yn, 23 Jan. 1885, *J. M. Delavay 1218*" (P); "In umbrosis silvarum ad Peetsao-lo, supra Mo-so-yn, 9 Apr. 1886" (not located); "In silvis ad Ta-long-tan prope Tapin-tze, 1800 m, 20 Jan. 1887, *J. M. Delavay 2313*" (P, US). I selected as lectotype *Delavay 1218* kept at P, because it is better preserved.

Key to the varieties of Ainsliaea pertyoides *in China*

1a. Leaves light green below (with long straight trichomes only) . . . . . 24a. var. *pertyoides*
1b. Leaves white-lanate below (with long straight trichomes and long crisped trichomes) . . . . . 24b. var. *albotomentosa*

Figure 35. *Ainsliaea pertyoides* Franch. A–F. *A. pertyoides* var. *pertyoides*. —A. Habit. —B. Capitulum. —C. Phyllaries. —D. Chasmogamous floret. —E. Stamen. —F. Style branches. G, H. *A. pertyoides* var. *albotomentosa* Beauverd. Foliar trichomes. Scale bars: A = 3 cm; B–D = 5 mm; E, F = 2 mm; G = 0.1 mm; H = 0.2 mm. Drawn from A–C, *Rock 4072* (US); D–H, *Delavay in 1888* (UC).

24a. Ainsliaea pertyoides Franch. var. **pertyoides.** Figure 35A–F.

Leaves green on both surfaces, slightly discolorous, with upper surface glabrous and lower surface reddish green, sparsely strigose, with long straight trichomes.

Distribution and phenology. Restricted to southwestern China (Yunnan). This variety was collected in forest in shady places at elevations between 1500 and 2000 m, in calcareous or sandy soils; flowering in February and March.

Representative specimens examined. CHINA. **Yunnan:** "Hu che kou, près de Mo so yn," 28 Jan. 1889, *J. M. Delavay s.n.* (S); "in silvis ad Ta-long-tan prope Tapin-tze," *J. M. Delavay 2313* (paratype of *Ainsliaea pertyoides*, P, US); Lou Kiuen, *F. Ducloux 256* (UC); "Infra vicum Hsinlung trans flumen Pudu-ho ad Septentr. urbis. Yünnanfu" [Kun-ming], *F. Handel-Mazzetti 511*, Diar. Nr. 120 (M, W); "Mengtze" [Mengsi], *A. Henry 9882A* (MO); "Szemeo" [Simao], *A. Henry 9882B* (MO, NY); mountains S of "Likiang" [Lijiang], near Hochin and Chiuho, *J. F. Rock 4072* (US).

24b. Ainsliaea pertyoides Franch. var. **albotomentosa** Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 384. 1909. TYPE: China. Yunnan: Yu-nan-sen, Feb. 1904, *F. Ducloux 254* (lectotype, designated here, UC!; duplicate, NY!). Figure 35G, H.

Ainsliaea ovalifolia Vaniot, Bull. Acad. Int. Géogr. Bot. 12: 119. 1903. *Ainsliaea pertyoides* f. *ovalifolia* (Vaniot) Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 384. 1909. *Ainsliaea pteropoda* DC. f. *ovalifolia* (Vaniot) H. Lév., Cat. Pl. Yun-Nan: 37. 1915. TYPE: China. Yunnan: Yu-nan-sen bois de la pagode de He-long-tan, 24 Feb. 1897, *Bodinier & Deblenne 78* (lectotype, designated here, K!; duplicate, W!).

Ainsliaea pertyoides var. *intermedia* Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 384. 1909. TYPE: China. Yunnan: Yun-nan-sen, à Lan-ngy-tsin près Lou lan, Jan. 1907, *F. Ducloux 296* (lectotype, designated here, NY!; duplicate, UC!).

Leaves strongly discolorous, with upper surface dark green, glabrous, and lower surface densely white-lanate, with long crisped trichomes mixed with long straight trichomes.

Distribution. China (Guizhou, Yunnan). Although this variety occurs within the range of var. *pertyoides*, it was collected from 2000 to 2600 m; flowering in January, March, April, September, and October.

Discussion. Two specimens from Yunnan are mentioned in the protologue of *Ainsliaea pertyoides* Franch. var. *albotomentosa*: "Yu-nan-sen. Feb. 1904. *F. Ducloux 254*" and "Yun-nan-sen. Mar. 1905. *E. E. Maire s.n.*" Because I was unable to locate the collection *E. E. Maire in 1905*, I chose *F. Ducloux 254*, kept at UC, as the lectotype of var. *albotomentosa*.

Because no herbaria were mentioned for the type specimens in the protologues of *Ainsliaea ovalifolia* and *A. pertyoides* var. *intermedia*, I propose the collections kept at K and NY, respectively, as lectotypes because they are better preserved.

Representative specimens examined. CHINA. **Guizhou:** "Kweichou," *J. Cavalerie 4212* (UC, W), *J. Cavalerie 7485* (W). **Yunnan:** "Yunnanfu" [Kun-ming], *J. Cavalerie 19* (S); Central-Yunnan, Xundian, *P. I. Chiou 042* (CM); Ta-long-tang, *J. M. Delavay in 1888* (UC); 12 Jan. 1889, *J. M. Delavay s.n.* (UC); Hu che kou, près de Mo so yn, 28 Jan. 1889, *J. M. Delavay s.n.* (UC); Ly Ma Teou, près Lou-Lan, *F. Ducloux 295* (NY, UC); "Lan-ngy-Asin," *F. Ducloux 2348* (P); environs de Hay-tien, *F. Ducloux 2418* (P); région de Kiao-Kia, *F. Ducloux 6028* (NY); environs de Yunnan-sen, *F. Ducloux 2271* (P); Prope vicum Sanyingpan ad septentr. urbis Yünnanfu, *F. Handel-Mazzetti 582*, Diar. Nr. 145 (W); Mi-lê district, *A. Henry 9926* (MO); Yunnan-sen, *E. E. Maire 102* (UC); San-tao-keou, *E. E. Maire 352* (W); Yunnan-sen, *E. E. Maire 1084, 2401* (UC); Montagneson Siao-bo, *E. E. Maire 2517* (NY, UC); Ma-li-ouan, Jan., s.d., *E. E. Maire s.n.* (W 16887); Yun-nan-sen, *E. E. Maire in 1905* (paratype of *A. pertyoides* var. *albotomentosa*, not seen).

25. Ainsliaea pingbianensis Y. C. Tseng, Acta Phytotax. Sin. 31: 365. 1993. TYPE: China. Yunnan: Pingbian, 1780 m, 6 May 1954, *P. Mao 4186* (holotype, IBSC not seen; isotype, IBSC photo LP!). Figure 36.

Plants perennial, herbaceous, 0.30–0.50 m tall; stems erect, unbranched below the inflorescence, leafy and strigose. Leaves basally clustered, rosulate, long-petiolate; petioles wingless, 4–11 cm, densely fulvo-strigose; leaf blades subcoriaceous, oblong to elliptic or oblong to lanceolate, 8–12 × 3–6 cm, apex obtuse, base rounded, margins shallowly denticulate and curved-ciliate; strigose on both surfaces with long straight trichomes, ca. 2.5–4 mm; palmati-pinnate, 3-nerved; cauline leaves short-petiolate, similar to the basal, gradually reduced upward to the base of the inflorescence. Capitula short-pedunculate, arranged in thyrsoid inflorescence; involucre 5- or 6-seriate, cylindrical, ca. 9 × 3–4 mm; phyllaries papyraceous, dorsally slightly pilose; outer phyllaries ovate, acuminate to acute, 1–2.5 × 0.9–1.5 mm; inner phyllaries ovate to lanceolate or ovate to elliptic, acuminate to acute, usually 3- to 5-nerved, 7–10 × 1–2.2 mm. Florets 3, hermaphrodite, chasmogamous and cleistogamous (at different seasons); *chasmogamous florets* (not seen, described from the original diagnosis) with corollas ligulate, ca. 9 mm, lobes ca. 4 mm. Achenes ca. 3.6 mm; pappus bristles ca. 7 mm; *cleistogamous florets* with corollas tubulose to pseudoligulate, ca. 4 mm, lobes ca. 0.4 mm (deeper

Figure 36. *Ainsliaea pingbianensis* Y. C. Tseng. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Phyllaries. —E. Cleistogamous floret. —F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 0.1 mm; C, D = 2 mm; E = 1 mm; F, G = 0.5 mm. Drawn from *Chen 15094* (MO).

slit ca. 0.6 mm); anthers ca. 1.7 mm, apical appendages short-apiculate, 0.5 mm, sagittate; tails smooth to slightly pilose, ca. 0.5 mm; style branches short, obtuse to acute, ca. 0.5 mm. Achenes oblong, attenuate at the base, ca. 4 mm, 10-nerved, densely pilose; pappus bristles 24 to 44, ca. 6 mm.

Distribution and phenology. Southern China (Guangdong, Sichuan, Yunnan); collected at elevations between 1300 and 1800 m; flowering in November and December (flower bud in July).

Relationships. *Ainsliaea pingbianensis* resembles *A. fulvipes*. Both species have leaves in a basal rosette, with leaf blades that are oblong to elliptic, petioles densely strigose, and capitula 3-flowered. However, the leaf blades of *A. pingbianensis* are larger (8–12 cm long vs. 3–7.5 cm), the style branches are obtuse to acute (vs. truncate), and the apical anther appendages are short-apiculate (vs. rounded).

Representative specimens examined. CHINA. **Guangdong:** Canton, *Guangdong 77 Team 5555* (MO). **Sichuan:** Kansei, *S. Q. Chen 15094* (MO); Kansei, *Z. Zhang 13248* (MO). **Yunnan:** Hekou, *W. Liu 634* (paratype, IBSC not seen); Malipo, *K. Feng 12790* (paratype, IBSC not seen).

26. Ainsliaea qianiana S. E. Freire, Novon 12: 453. 2002. TYPE: China. Yunnan: Colline de Kin-tchong-chan, s.d., 2990 m, *E. E. Maire 2529* (holotype, W!; isotypes, NY!, UC!).

Plants perennial, herbaceous, 0.50–0.60 m tall; stems erect, unbranched, with few leaves much reduced, moderately strigose. Leaves basally clustered, rosulate, long-petiolate; petioles winged, 10–11 × 0.8–1 cm (at the upper point); leaf blades slightly fleshy, elliptic or elliptic to ovate, 7.5–8.5 × 3.5–3.8 cm, apex acute to subobtuse, base abruptly rounded and decurrent into the petiole, margins denticulate, with long straight trichomes, 0.6–1 mm long; upper and lower surfaces glabrous, the surface paler below; palmati-pinnate, 3-nerved; cauline leaves alternate, short-petiolate to sessile, linear to elliptic, ca. 2 × 0.5 cm. Capitula sessile, arranged in a stachyoid inflorescence; involucre 5- to 7-seriate, cylindrical, 12–14.5 × 3–4 mm; phyllaries papyraceous, purple, glabrous; outer phyllaries linear to ovate, acuminate, 2.8–3 × 0.8–1 mm; inner phyllaries linear to ovate or linear to elliptic, acuminate to acute, midvein dark, 11–13 × 1.2–2 mm. Florets 3, hermaphrodite, chasmogamous; corollas pink, lobes 3.5 mm (deeper slit ca. 5.5 mm), tube 4.5 mm; anthers ca. 6 mm, apical appendages apiculate, 1–1.5 mm, sagittate; tails ca. 1.3 mm, shortly pilose; style branches short-bilobate, rounded, ca. 0.25 mm. Achenes oblong, 3–4 mm, 10-nerved, densely pilose; pappus bristles ca. 39, 6–7 mm.

Distribution and phenology. China (Sichuan, Yunnan); collected at elevations between 2990 and 3640 m; flowering in October.

Relationships. This species is characterized by its apiculate anther appendages, rosulate leaves with winged petioles, and 3-flowered capitula. *Ainsliaea qianiana* exhibits some likeness to *A. macrocephala* in its habit and capitula. The latter species is amply distinguished by its rounded apical anther appendages and its pilose phyllaries and leaves. *Ainsliaea qianiana* also resembles *A. yunnanensis*, but *A. qianiana* may be distinguished by its apiculate apical anther appendages (vs. rounded) and its glabrous or subglabrous leaves (vs. strigose).

Observation of the type material. There are two other sheets from China, Yunnan, Colline de Kin-tchong-chan (*E. E. Maire 2529* at NY and UC), which correspond to *Ainsliaea yunnanensis*.

Representative specimens examined. CHINA. **Sichuan:** In regionis temperate inter oppidum Yenyuen et flumen Yalung, *F. Handel-Mazzetti 5383*, Diar. No. 896 (W).

27. Ainsliaea ramosa Hemsl., J. Linn. Soc. Bot. 23: 171. 1888. TYPE: China. Hubei: Hupeh, "Nanto and mountains to the northward," year 1885–1888, *A. Henry 4422* (holotype, K not seen; isotype, US!). Figure 37.

Plants perennial, herbaceous, 0.30–0.55 m tall; stems erect, unbranched below the inflorescence, naked, with few leaves reduced or scattered leafy, strigose. Leaves basally clustered, rosulate, long-petiolate with petioles wingless, strigose, 4–8 cm; leaf blades subcoriaceous, ovate to oblong, 7.5–12.5 × 3.5–6.5 cm, acute to subobtuse at the apex, base deeply cordate, margins obscurely denticulate; upper surface sparsely strigose, and lower surface paler, red strigose, especially on the nerves (with long straight trichomes 1–1.5 mm); palmati-pinnate, 3- to 5-nerved. Capitula sessile or shortly pedunculate, arranged in thyrsoid inflorescence; involucre 5- or 6-seriate, cylindrical, ca. 10 × 7 mm broad; phyllaries papyraceous, purple, glabrous; outer phyllaries ovate, acute to acuminate, ca. 1.5 × 1 mm; inner phyllaries linear to elliptic, acute to acuminate, 3- to 5-nerved, ca. 10 × 0.9 mm. Florets 3, hermaphrodite, chasmogamous and cleistogamous (at different seasons); corollas white; *chasmogamous florets* with corollas ligulate, ca. 7 mm, lobes ca. 3 mm (deeper slit ca. 4 mm), tube ca. 3 mm; anthers ca. 4 mm, apical appendages apiculate, 0.5 mm, tails ca. 1.5 mm, smooth to slightly pilose; style branches short, rounded, ca. 0.4 mm. Achenes oblong, 1.5–2 mm, 10-nerved, densely pilose; pappus bristles 35

Figure 37. *Ainsliaea ramosa* Hemsl. —A. Habit. —B. Foliar trichome. —C. Phyllaries. —D. Capitulum. —E–H. Cleistogamous floret: E. stamen; F. style branches; G. corolla; H. achene with pappus. —I. Chasmogamous floret. Scale bars: A = 3 cm; B = 0.1 mm; C = 2 mm; D, I = 5 mm; E = 0.8 mm; F = 1 mm; G = 2 mm; H = 1.5 mm. Drawn from A–H, *Wang 10279* (MO); I, *Tsang 20084* (US).

to 37, ca. 5 mm; *cleistogamous florets* with corollas tubulose to pseudoligulate, ca. 4.5 mm, 5-lobed (deeper slit ca. 0.3 mm); anthers ca. 3 mm; style branches ca. 0.4 mm. Achenes ca. 2.5 mm, pappus bristles ca. 6 mm.

Distribution and phenology. Central and southern China (Guangdong, Guangxi, Hubei); collected on rocky hills; flowering in April and October, fruiting in January.

Relationships. *Ainsliaea ramosa* most resembles the species complex with *A. caveleriei*, *A. fragrans*, and *A. rubrinervis*. This complex and *A. ramosa* share leaves ovate to oblong, deeply cordate at the base, with villous petioles. However, *A. ramosa* may be differentiated by its apical anther appendages that are apiculate, and its phyllaries that are glabrous.

Representative specimens examined. CHINA. **Guangdong:** "Kwangtung," Naam Kwan Shan (Tsengshing District), *W. T. Tsang 20084* (MO, NY, US, W). **Guangxi:** Xiangzhou Xian, *C. Wang 40279* (MO); Xiangzhou Xian, *C. Wang 40798* (MO).

28. Ainsliaea reflexa Merr., in Philipp. J. Sci. 1, suppl. 3: 242. 1906. TYPE: Philippines. Luzon: Lepanto, Mt. Data, 4 Nov. 1905, *E. D. Merrill 4571* (holotype, NY!). Figure 38.

Ainsliaea longipetiolata Merr., Pap. Michigan Acad. Sci. 24: 91. 1939. Syn. nov. TYPE: Indonesia. Sumatra: E Coast, Karo Highlands, summit of Deleng Pintoe, 11 Jun. 1928, *C. Hamel & Rhamat Si Toroes 615* (holotype, NY!; isotype, MICH not seen).

Ainsliaea reflexa var. *lobbiana* Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 2: 37. 1910. Syn. nov. TYPE: Philippines, s.d., *Th. Lobb 447* (holotype, K not seen; isotypes, W!, K photo LP!).

Ainsliaea tonkinensis Merr., Univ. Calif. Publ. Bot. 13: 142. 1926. Syn. nov. TYPE: Vietnam. Tonkin: Chapa, April 1925, *A. Pételot 1767* (holotype, UC!).

Plants perennial, herbaceous, 0.10–0.60 m tall; stems erect, unbranched, with few leaves much reduced, loosely strigose. Leaves basally clustered, rosulate, long-petiolate; petioles flat, winged on upper half to practically wingless, sparsely strigose, scattered to much exceeding the lamina, 3–9 cm; leaf blades membranous, ovate or oblong to elliptic, 2–7 × 1.2–4 cm, acute at the apex, abruptly contracted and cuneate at the base, margins slightly wavy, with distant, short-spinulose teeth on each side of the midrib; deep green and subglabrous above, paler below and sparsely strigose, with veins somewhat more densely pubescent than the rest of abaxial surface, with few long straight trichomes, 1–2.5 mm; palmati-pinnate, 3-nerved. Capitula sessile or short-pedunculate, arranged in a stachyoid inflorescence; peduncles 2–3 mm long; involucre 5- or 6-seriate, cylindrical to obdeltate, 6–12 × 2–5 mm; phyllaries papyraceous, glabrous to sparsely strigose above; outer phyllaries ovate, acute, 1.5–2 × 0.7–0.8 mm; inner phyllaries linear to lanceolate, acute, midvein dark, 7–12 × 1–1.3 mm. Florets 3, hermaphrodite, chasmogamous (with or more commonly without pappus) and cleistogamous (in single plant or at different season); corollas white or pale pink; *chasmogamous florets* with corolla ligulate, 5.5–6.5 mm, lobes 2–2.5 mm (deeper slit 2.7–3.5 mm), tube 2.7–3 mm; anthers 2.5–3 mm, apical appendages rounded, ca. 0.5 mm, sagittate; tails ca. 1 mm, shortly pilose; style branches short, truncate, 0.4–1 mm. Achenes oblong to elliptic, 1.5–2.5 mm, 10-nerved, pilose; pappus bristles 0(1, 2 or 8) or ca. 26, uniseriate, 4–4.5 mm; *cleistogamous florets* with corolla tubulose to pseudoligulate, 4-lobed, 2.2–3.5 mm, lobes ca. 0.2 mm (deeper slit ca. 0.4 mm); anthers ca. 1.2 mm; style branches ca. 0.5 mm. Achenes pilose, 3–5 mm; pappus bristles ca. 30, 4.5–7 mm.

Distribution and phenology. Southern China (Guangdong, Yunnan), Indonesia (Sumatra), Philippines, Taiwan, and southern Vietnam. It was collected in shaded mountain forest and in moist places along rivers and streams, or sunny grass fields, at elevations between 1800 and 3450 m (Fig. 7A); flowering and fruiting throughout the year.

Discussion. According to the protologue, *Ainsliaea tonkinensis* and *A. longipetiolata* were based on their long petiolate leaves (petioles 2–4 cm and 5–8 cm, respectively), with leaf blades oblanceolate and ovate to elliptic, respectively. After examining the type materials and subsequent collections, I considered these characters to be within the range of variation for *A. reflexa*.

Beauverd (1910) established the var. *lobbiana* because of its much smaller leaves, i.e., leaf blades 1–2 × 0.5–1.2 cm (vs. 2.5–6 × 1–2 cm in the protologue of *Ainsliaea reflexa*) and capitula sessile (vs. shortly pedunculate with peduncles of 2–3 mm in the protologue of *A. reflexa*). However, examination of the type material of var. *lobbiana* at W reveals that the smaller leaves are 2 × 1.2 cm. Because *A. reflexa* var. *lobbiana* does not appear to differ significantly from typical *A. reflexa*, I propose to place the var. *lobbiana* into synonymy of *A. reflexa*.

Vernacular name. Taiwan-haguma (Yamamoto, 1936).

Relationships. *Ainsliaea reflexa* is probably closely related to *A. latifolia* var. *taiwanensis*. Both taxa have leaves long-petiolate and achenes densely pilose. *Ainsliaea reflexa* is distinguished from *A. latifolia* var.

Figure 38. *Ainsliaea reflexa* Merr. A. Habit. B. Foliar trichome. C–F. Leaves from different specimens. —G. Phyllaries. —H. Style branches of chasmogamous floret. —I. Stamen of chasmogamous floret. —J. Chasmogamous floret epappose. —K. Chasmogamous floret pappose. —L. Stamen of cleistogamous floret. —M. Style branches of cleistogamous floret. —N. Cleistogamous floret. Scale bars: A = 3 cm; B = 0.1 mm; C–F = 3.5 cm; G = 2 mm; H, I = 1 mm; J, K = 2 mm; L, M = 1 mm; N = 2 mm. Drawn from A, B, G–N, *Ramos & Edaño 14917* (UC); C, the holotype *Hamel & Rhamat 615* (NY); D, the holotype *Merrill 4571* (NY); E, the isotype *Lobb 447* (W); F, *Suzuki 1450* (MO).

taiwanensis by its petioles that are only winged on the upper half to practically wingless (vs. petioles broadly winged). *Ainsliaea reflexa* also resembles *A. henryi* in its ovate or oblong leaf blades and winged on the upper half to practically wingless petioles, but *A. reflexa* differs from *A. henryi* by its achenes densely pilose (vs. glabrous or apically pilose).

Observations. Plants with flower stems few branched rather than single occur sporadically in northern Vietnam and Sumatra. A few examples of these plants are *A. Pételot 2067* (NY, US), *3680* (C), and *O. Hagerup in 1916–1917* (C). These specimens resemble *Ainsliaea glumacea*, but they differ from it by their leaf bases contracted into the petiole (vs. uniformly narrowed).

The composition floret varies on a single plant from all cleistogamous, cleistogamous and chasmogamous, to all chasmogamous. This variation seems to coincide with the time of collection. In fact, according to the materials examined, i.e., between September and January, the florets are cleistogamous; from February to April, the plants have both florets, cleistogamous and chasmogamous (with or without pappus); and finally from May to July plants with chasmogamous florets (without pappus) are found. However, there is a period (February to April) in which the three possibilities can be found.

Representative specimens examined. CHINA. **Guangdong ("Kwangtung"):** Hainan, *Wang 35545, 35546* (NY). **Yunnan:** Yangtze watershed, western slopes of Likiang Snow Range, *J. F. Rock 4216* (US, W). INDONESIA. **Sumatra:** trail up volcano "Sinaboeng" [Sinabung], *W. N. & C. M. Bangham 1191* (NY); Toba, *O. Hagerup in 1916–1917* (C); Gunung Leuser Nature Reserve Atjeh, North Sumatra Ketambe, Gunung, ca. 40 km NW of Kutatjane, 8–15 km SW from the mouth of Lau Ketambe, Camp 9, *W. J. J. O. de Wilde & B. E. E. de Wilde-Duyfjes 14245* (US), 10 km NE of Kampung, Seldok (Alas Valley), ca. 25 km N of Kutatjane, *W. J. J. O. de Wilde & B. E. E. de Wilde-Duyfjes 15196* (MO, US); Gunung Leuser Nature Reserve, Atjeh, N Sumatra, Camp 4-5, Climbing Gunung Mamas, ca. 17 km SW from the mouth of Lau Ketambe, ca. 30 km NW of Kutatjane, *W. J. J. O. de Wilde & B. E. E. de Wilde-Duyfjes 16734* (MO); Mt. Leuser, Bivonsa No. 4, *S. Dillon Ripley & F. A. Ulmer 43* (PH); Mt. Leuser, Bivonsa No. 7, *S. Dillon Ripley & F. A. Ulmer 71* (PH); vicinity of Taloen na Oeli, Toba (E of Dolok Si Manoek-manoek, near headwaters of Aek Mandosi: topographic sheet 29, NW quarter), *Rahmat Si Boeea 10132* (NY); residency of Tapianoeli, headwaters of Aek Mandosi, Toba (vicinity of Taloen na Oeli, near Toba-Asahan boundary: topographic sheet 29, W half, N of center), *Si Boeea Rahmat 10451* (NY). PHILIPPINES. **Luzon:** Bontoc, Pauai, Benguet to Mt. Data, *M. S. Clemens 16277* (UC); Benguet, *M. S. Clemens 17256b* (UC); Mt. Pulog, Benguet, *M. S. Clemens in 1925* (UC 268216); Mt. Data, Bontoc, *M. S. Clemens in 1928* (UC 374850), *M. S. Clemens 18906* (UC); Benguet, Baguio, *A. D. E. Elmer 8363* (MO, NY, PH, W); Benguet, Baguio, *A. D. E. Elmer 14193* (C, MO, NY, US, W); Benguet, Baguio, *A. D. E. Elmer 14293* (C, MO, NY, US, W); Mt. "Banajao," *F. W. Foxworthy herb. 2400* (US); Mt. Banaho, *F. C. Gates et al. 7262* (NY); Mt. Pulog, *M. Jacobs 7081* (C, CANB, MO); Luzon central, Benguet, *A. Loher 3621* (M); "Philippine Islands," *A. Loher 6474* (M); Rizal Prov., *A. Loher 14045* (M, UC); Benguet, Mt. Sto. Tomas, *McClure 16069* (UC); summit of Mt. Santo Tomas, *E. A. Mearns 19, 20* (US); Laguna, Mt. Banajao, Mar. 1911, *E. D. Merrill 734* (M, US); Benguet, *E. D. Merrrill 835* (M, US); Benguet, Mt. Tonglon, *E. D. Merrill 4824* (NY); Benguet, Mt. Pauai, *E. Quisumbing & M. Sulit 6240* herb. 82335 (NY); Bontoc, Mt. Polis, *M. Ramos & G. Edaño 37614* (UC, US); Mt. Data, Lepanto, *M. Ramos & G. Edaño 40191* (UC, US, W); Lepanto, Mt. Pangualaytacay, *M. Ramos & G. Edaño 40469* (US); Benguet, Mt. Pulog, *M. Ramos & G. Edaño 44917* (NY, UC); Benguet, Pauai, *J. K. Santos 32047* (NY); Mountain Prov., Bontoc, Barrio Agawa, Besao, near summit of Mt. Masbit, *J. Vera Santos 5436* (US); N Luzon, Benguet (Prov. Union), Mt. Santo Tomás, *R. S. Williams 1434* (NY, US). TAIWAN. **Ilan:** Mt. Nan-liu, Co. Ilan, *C. C. Chuang & T. Kao 2526*, p.p. (AAU). **Kaohsiung:** Taoyuan Hsiang, Paoshan Village, rd. from Shelter to end of Chuyunshan Forest Rd., *H. L. Ho et al. 879* (HAST, MO); Toayan Co., Palin to Tamanshan, *K. C. Yang 1318* (NY). **Nantou:** Jenai Hsiang, en route from Yunhai to Tienchih, *H. L. Ho et al. 611* (MO); Chitou to Chiti, *T. Koyama 8643* (NY). **Pingtung:** Wutai Hsiang, en route from entrance to Payutzu, *C. H. Lin & H. F. Yen 348* (US). **Unknown:** Mt. A-Li, Co. Chiayi, *C. C. Chuang & M. T. Kao 4198* (PH); Mt. Arisan, *R. Kanehira & S. Sasaki 21644* (UC); Mt. Taipinshan, *H. Keng K1214* (US); Arisan, *J. Linsley Gressitt* 195 (NY, S); Mt. Taiheizan, *S. Suzuki in 1929* (US); Mt. Taiheizan, *S. Suzuki 1450* (MO); Mt. Kiraisyu-namp, *S. Suzuki 2256* (US); Arisan, *S. Suzuki & Y. Kudo in 1928* (S); Mt. Chiagshui, Co. Hualien, *M. Teraya 459*, p.p. (CM). VIETNAM. **Kontum:** SW slopes of Ngoc Linh mountain system, *L. Averyamov et al. VH 471* (AAU). **Tonkin:** Cha Pa, *A. Pételot 2067* (NY, US); Cha Pa, *A. Pételot 3680* (C, NY).

29. Ainsliaea rubrinervis C. C. Chang, Sinensia 4(8): 226. 1934. TYPE: China. Sichuan: Kwanhsien, 3000–3600 ft., 7 July 1928, *W. P. Fang 2076* (holotype, N not seen; isotype, NY!). Figure 39.

Plants perennial, herbaceous, 0.15–0.60 m tall; stems erect, unbranched, naked, with few leaves reduced, strigose. Leaves basally clustered, rosulate, long-petiolate with petioles wingless, strigose, (1.5)6–7(11) cm; leaf blades subcoriaceous, ovate to oblong, (3)7–10.5 × (1.5)3–3.5 cm, acute at the apex, base deeply cordate, margins obscurely denticulate; upper surface sparsely strigose, lower surface whitish green, and red-strigose with long straight trichomes ca. 1.5 mm; palmati-pinnate, 3- to 5-nerved, veins prominent and red on lower surface. Capitula sessile or short-pedunculate, arranged in stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, 8–10 × ca. 4 mm; phyllaries papyraceous, glabrous; outer phyllaries ovate, acute, 2–3 × 1–1.5 mm; inner phyllaries linear to elliptic, acuminate, 3-nerved, 8–9 × ca. 2 mm. Florets 3, hermaphrodite, chasmogamous; corollas white, ligulate, ca. 10 mm, lobes ca. 3.5–4 mm (deep slit ca. 6 mm), tube ca. 4 mm;

Figure 39. *Ainsliaea rubrinervis* C. C. Chang. —A. Habit. —B. Capitulum. —C. Phyllaries. —D. Foliar trichome. —E. Style branches. —F. Stamen. G. Chasmogamous floret. Scale bars: A = 3 cm; B = 5 mm; C = 2 mm; D = 0.1 mm; E, F = 0.1 mm; G = 2 mm. Drawn from the isotype *Fang 2076* (NY).

anthers purple, ca. 5 mm, apical appendages apiculate, ca. 1 mm, sagittate, tails 1–1.5 mm, shortly pilose; style branches short, rounded, 0.5 mm. Achenes oblong-elliptic, 2–3.5 mm, 10-nerved, densely pilose; pappus bristles ca. 39, 7–7.5 mm.

Distribution and phenology. *Ainsliaea rubrinervis* at present is only known from the type collection from central China (Sichuan) in woods, between 900 and 920 m; flowering in July.

Relationships. *Ainsliaea rubrinervis* most resembles the species complex constituted by *A. cavaleriei*, *A. ramosa*, and *A. fragrans*. This complex and *A. rubrinervis* share leaves that are ovate to oblong, deeply cordate at the base, with villose petioles. *Ainsliaea rubrinervis* may be distinguished by its apical anther appendages apiculate, phyllaries glabrous, and its leaf veins violet beneath.

30. Ainsliaea smithii Mattf., Acta Horti Gothob. 8: 79. 1933. TYPE: China. Sichuan: Sze-chuan, Drogochi, 3400 m, 26 Sep. 1922, *H. Smith 4698* (type fragm., W!). Figure 40.

Plants perennial? (rhizome unknown), herbaceous, ca. 0.40 m tall; stems erect, leafy, unbranched below the inflorescence, lanuginose. Leaves basally clustered? (rosette unknown), long-petiolate with petioles broadly winged, slightly narrowed to the base, 8.5–11 × 1.2 (at the upper point) cm; leaf blades chartaceous, ovate to ovate-deltoid, 10–12 × 8–9.5 cm, apex acute to acuminate, cordate at the base, margins obscurely denticulate; sparsely lanate on both surfaces with short glandular biseriate trichomes, and long crisped trichomes; palmati-pinnate, 3- to 5-nerved; cauline leaves alternate, short-petiolate, ovate, gradually reduced upward becoming linear to lanceolate, (1)4(7.5) × (0.2)1(3) cm. Capitula short-pedunculate, arranged in thyrsoid inflorescence; involucre 5- or 6-seriate, cylindrical, 10–12 × 2.5–3 mm; phyllaries papyraceous; outer phyllaries ovate, acuminate, ca. 3 × 1 mm, glabrous; inner phyllaries linear to elliptic, acute to acuminate, midvein dark, ca. 11 × 0.7 mm, dorsally with short glandular trichomes. Florets 3, hermaphrodite, cleistogamous; corolla tubulose to pseudoligulate, ca. 6 mm, lobes ca. 0.8 mm (deeper slit ca. 1.2 mm); anthers ca. 1.9 mm, apical appendages rounded, ca. 0.4 mm, sagittate; tails ca. 0.7 mm, smooth to slightly pilose; style branches short, rounded, ca. 0.3 mm. Achenes oblong-obovate, ca. 2.5 mm, 10-nerved, densely pilose; pappus bristles ca. 32, ca. 8 mm.

Distribution and phenology. At present *Ainsliaea smithii* is only known from the type collection, China (Sichuan); flowering in September.

Discussion. No other material was mentioned by Mattfeld, and I was unable to find another collection in order to select a neotype.

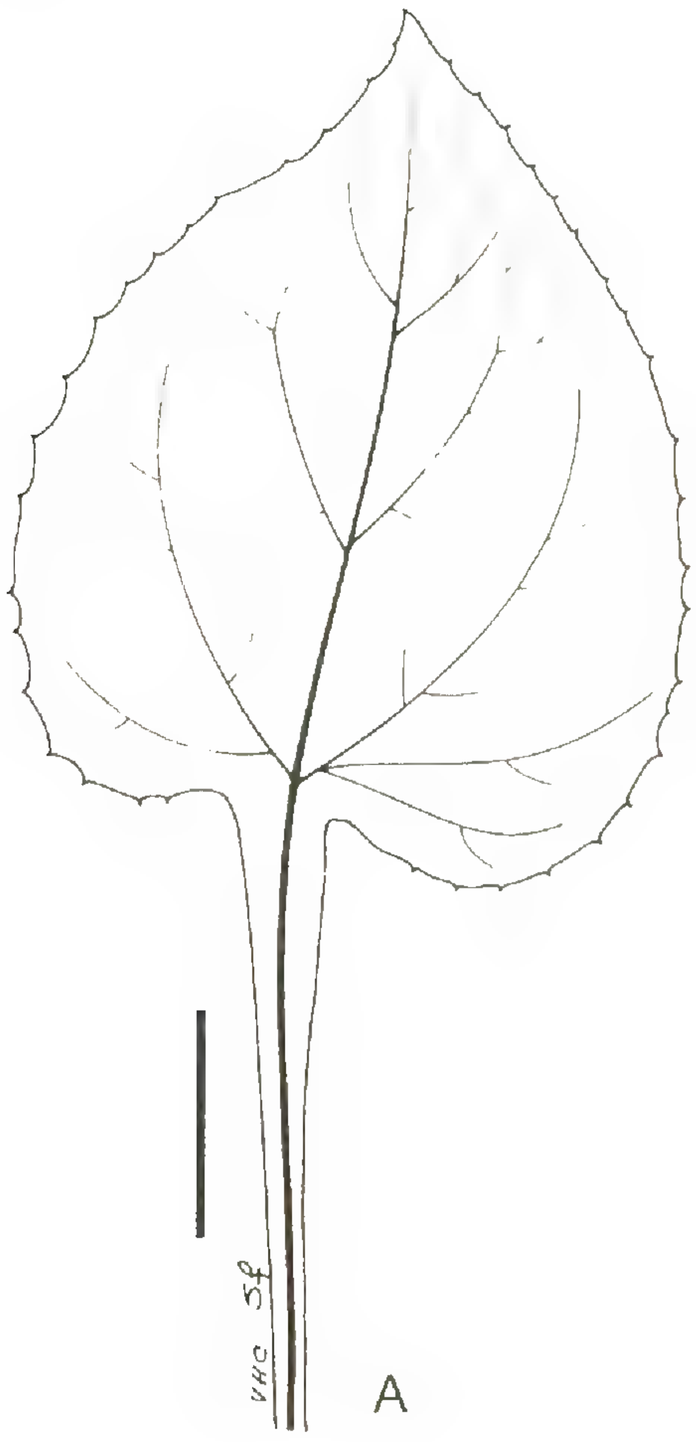
Relationships. *Ainsliaea smithii* is closely related to *A. bonatii*. Both species have leaves with winged petioles and cordate leaf blades. They differ only in its phyllaries, i.e., dorsally glandular and acuminate at the apex in *A. smithii* vs. apically strigose and acute at the apex in *A. bonatii*.

31. Ainsliaea spanocephala Y. C. Tseng, Acta Phytotax. Sin. 26: 74. 1988. TYPE: Thailand. Loei: Phu Luang, Phu Ruea, Wildlife Sanc. Stat., 1450 m, 19 Feb. 1983, *H. Koyama, H. Terao & W. Thawatchai 33699* (holotype, KUN not seen; isotypes, BKF!, GH!, KYO!). Figure 41.

Plants perennial, herbaceous, 0.45–0.90 m tall; stems erect, unbranched below the inflorescence, naked and glandulose-strigose. Leaves basally clustered, rosulate, long-petiolate; petioles wingless, conspicuously villose, (2)5–12 cm; leaf blades subcoriaceous, elliptic or ovate to elliptic, 5–14 × 2–5.5 cm, acute to obtuse at the apex, base rounded, margins obscurely denticulate and pilose, upper surface sparsely strigose, lower surface strigose, with long 3-seriate straight trichomes; palmati-pinnate, 3-nerved. Capitula long-pedunculate, arranged in thyrsoid inflorescences; involucre 4- or 5-seriate, cylindrical, 12–14 × 5–6 mm; phyllaries papyraceous, dorsally with glandular trichomes; outer phyllaries ovate, acute, 2–3 × 0.8–1.5 mm; inner phyllaries linear to lanceolate, acute, 3-nerved, 11–14 × 1.5–3 mm. Florets 5, hermaphrodite, chasmogamous; corollas white, ligulate, 12–16 mm, lobes 5–8 mm (deeper slit 8–10.5 mm), tube 4–5.5 mm; anthers purple, 7–8 mm, apical appendages apiculate, ca. 1 mm, sagittate; tails 1.2–2 mm, shortly pilose; style branches short, truncate, ca. 0.5 mm. Achenes oblong, attenuate at the base, 2–4.5 mm, 10-nerved, densely red-pilose; pappus bristles ca. 36, 9–11 mm.

Distribution and phenology. It was collected in evergreen forest of central Thailand (Loei), commonly in shady spots along streams in lower mountains, at elevations between 1150 and 1530 m (Fig. 3B); flowering from December to February.

Discussion. *Ainsliaea brandisiana* sensu Koyama (in Acta Phytotax. Geobot. 32(1-4): 60, fig. 2, 1981) corresponds to *A. spanocephala* because the materials mentioned by Koyama for *A. brandisiana* ("Henipman 3608" and "Bunchuai 1501") have leaves that are rounded at the base.



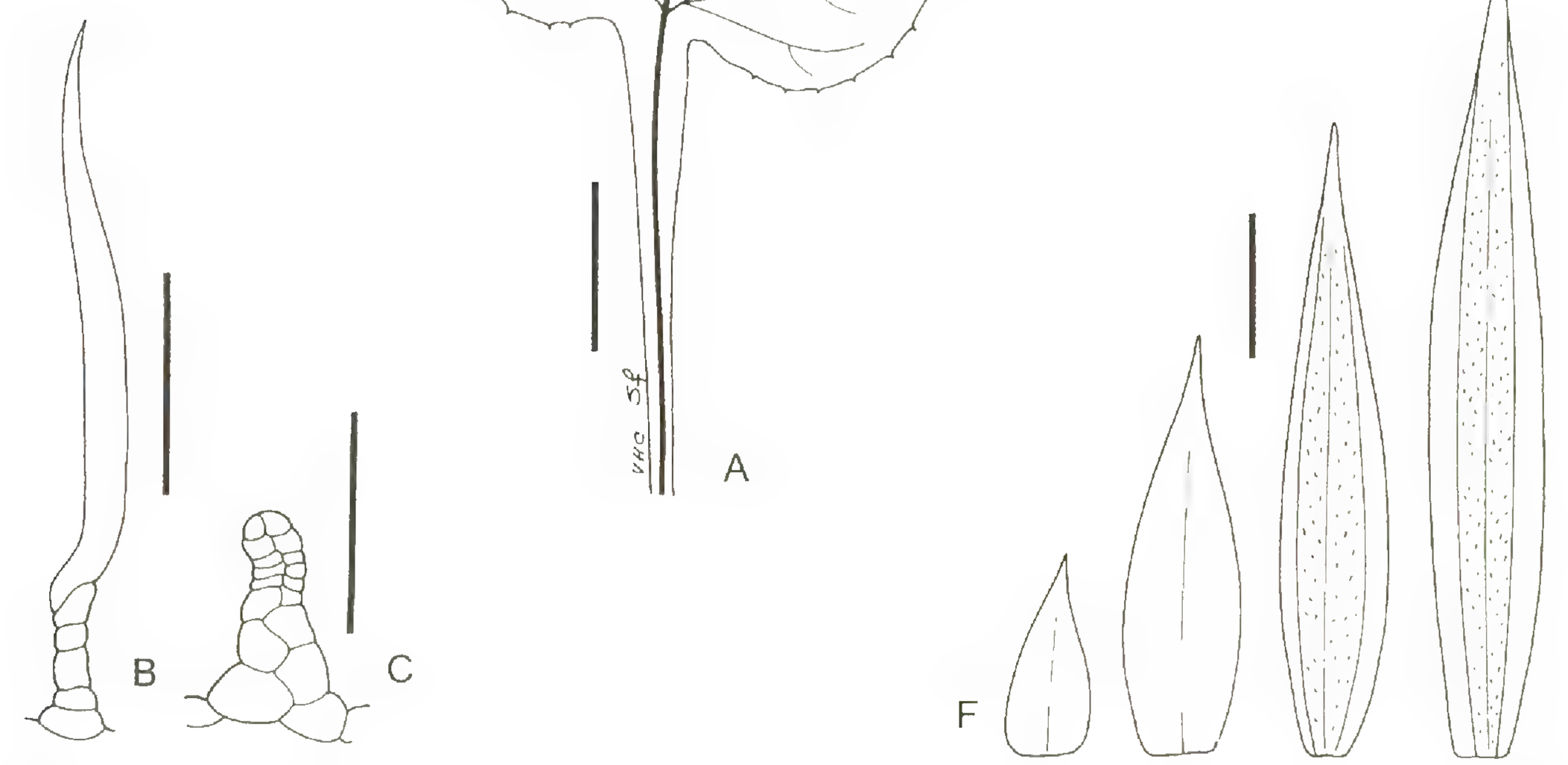


Figure 40. *Ainsliaea smithii* Mattf. A. Basal leaf. —B, C. Leaf trichomes. —D. Fragment of the inflorescence. E. Capitulum. —F. Phyllaries. —G–J. Cleistogamous floret: G, corolla; H, stamen; I, style branches; J, achene with pappus. Scale bars: A, D = 3 cm; B, C = 0.1 mm; E = 5 mm; F, J = 2 mm; G = 1 mm; H, I = 0.5 mm. Drawn from the type *Smith 4698* (W).

Figure 41. *Ainsliaea spanocephala* Y. C. Tseng. —A. Habit. —B. Capitulum. —C. Glandular trichome of phyllaries. —D. Foliar trichome. —E. Chasmogamous floret. —F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 5 mm; C = 0.05 mm; D = 0.15 mm; E = 5 mm; F = 2 mm; G = 1 mm. Drawn from A, the paratype *Hennipman 3608* (C); B, E–G, the paratype *Hennipman 3608* (BKF); C, D, *Niyomdham & Vidal 438* (C).

Relationships. *Ainsliaea spanocephala* is closely related to *A. brandisiana* Kurz. Both species have capitula with five florets and similar 3-seriate foliar trichomes. They are different only in the leaf base, rounded and cordate, respectively.

Representative specimens examined. THAILAND. **Loei:** Phu Luang, *K. Bunchuai 1501* (BKF); Phu Luang, Phu Ruea, Wildlife Sanc. Stat., *E. Hennipman 3608* (paratypes BKF, C, KYO); Phu Luang, Phu Ruea distr., Wildlife Sanctuary, *H. Koyama et al. T-31605, T-31642* (BKF, KYO); Phu Luang, *C. Niyomdham & E. Vidal 438* (AAU, BKF, C); Phu Luang, Wildlife Sanctuary, *T. Smitinand in 1982* (BKF 077323).

32. Ainsliaea spicata Vaniot, Bull. Acad. Int. Géog. Bot. 12: 117. 1903. TYPE: China. Yunnan: "Environs de Yun-nan-sen," 28 Jan. 1897, *F. Ducloux 9D* (holotype, E!; isotype, W!). Figure 42.

Ainsliaea pteropoda var. *obovata* Franch., J. Bot. (Desvaux) 2: 69. 1888. *Ainsliaea latifolia* var. *obovata* (Franch.) Grierson & Lauener, Notes Roy. Bot. Gard. Edinburgh 34: 385. 1976. TYPE: China. Yunnan: "Les bois de Mo che tchin au-dessus de Tapin-tze," 1800 m, 15 Jan. 1886, *J. M. Delavay 1921A* (type, P!).

Plants perennial, herbaceous, 0.18–0.60 m tall; stems erect, unbranched, naked, with few leaves reduced or more rarely leafy, lanuginose. Leaves basally clustered, rosulate, subsessile or shortly petiolate with petioles wingless, 4–7(10) mm; leaf blades chartaceous to slightly fleshy, obovate or obovate to pandurate, 5–13 × 2.5–5 cm, obtuse to subobtuse at the apex, base rounded abruptly narrowed onto the petiole, margins obscurely denticulate; upper surface sparsely strigose and lower surface strigose-lanate (with two types of trichomes, long crisped and long straight) or strigose (with long straight trichomes only); palmati-pinnate, 3-nerved; cauline leaves alternate, short-petiolate to sessile, ovate or ovate to pandurate. Capitula sessile or short-pedunculate, arranged in a stachyoid inflorescence; involucre 5- or 6-seriate, cylindrical, 8–12 × ca. 4 mm; phyllaries papyraceous, purple; outer phyllaries ovate, acute, ca. 3 × 1.3 mm, glabrous; inner phyllaries linear to elliptic, acute, midvein dark, ca. 12 × 1.4 mm, glabrous or short-pilose above. Florets 3, hermaphrodite, chasmogamous and cleistogamous (at different seasons or in single plant); corollas pink or white to pink; *chasmogamous florets* with corollas ligulate, 13–13.5 mm, lobes 6–6.5 mm (deeper slit ca. 7.5 mm), tube 5.5–6 mm; anthers ca. 5.5 mm, apical appendages rounded, ca. 1 mm, sagittate; tails 1.5–1.8 mm, shortly pilose; style branches short, truncate, ca. 0.2 mm. Achenes oblong, 2–3 mm, 10-nerved, densely pilose; pappus bristles 30 to 40, ca. 7 mm (sometimes absent); *cleistogamous florets* with corollas tubulose to pseudoligulate, 4–4.5 mm, 4-lobed (deeper slit ca. 0.3 mm); anthers ca. 1.5 mm; style branches 0.7–0.8 mm. Achenes 4-5 mm; pappus bristles 7–8 mm.

Distribution and phenology. Dry pebbly ground in light shade on sandy soils, between 1050 and 3700 m, from Bangladesh, Bhutan, China (Guizhou, Sichuan, Yunnan), India, and Thailand (Fig. 7B); collected in flower from January to March, in May, and from October to December.

Relationships. *Ainsliaea spicata* most resembles *A. latifolia*. Both have leaves basally clustered but may be differentiated by their leaves. *Ainsliaea latifolia* has ovate leaves, with long winged petioles, whereas *A. spicata* has obovate or obovate to pandurate leaves, subsessile or with short wingless petioles.

Representative specimens examined. BANGLADESH. "East Bengal," *Griffit 3331* (C, M). BHUTAN. No further data, s. coll. (W7212). CHINA. **Guizhou:** "Kouy-Tchéou" [Kwetschou], environs de "Kouy-Yang" [Guiyang, Kweyang], *R. P. Bodinier 1584* (NY). **Sichuan:** Huili, *F. Handel-Mazzetti 864*, Diar. Nr. 213 (W). **Yunnan:** "Yünnanfu" [Kun-ming], *J. Cavalerie 21* (S, W); Yünnanfu, ad templum Schili-ngan, *F. Handel-Mazzetti 293* Diar. Nr. 56 (W); in montis Taohwa-schan prope oppidum Beyendjing medium inter "Tschuhsiung" [Tsuyung] et Yungbei, Tschischingai, *F. Handel-Mazzetti 6255*, Diar. Nr. 1111 (W); "Mengtse" [Mengzi], s.d., *A. Henry 9294* (MO), *9294A* (NY) *9294B* (NY); "Szemao" [Simao], *A. Henry 12763A* (NY); "Jingdong" [Jinghong] Co., Mt. Tamati-houshan, *M. K. Li 0724* (HITBC); Tong-tchouan, *E. E. Maire* 3225 (NY, UC, US, W); Tong-chuan, Mar., *E. E. Maire s.n.* (W7135); NE-Yunnan, s.d., *E. E. Maire s.n.* (W7228); Jingdong Co., Xujiaba, 7 May 1981, s. coll. (HITBC 071883). **Unknown:** "Plants of E Tibet and SW China, Yengyueh," *G. Forrest 7865* (S). INDIA. **Assam:** Shillong, *C. B. Clarke 37392* (US); Khasia, *J. D. Hooker & T. Thomson s.n.* (NY, S, W1257); on way to Shillong, *S. L. Kapoort 75623* (MO). **Manipur:** Manipur, Ukrul Nagaberge, *A. Meebold 6805* (W). THAILAND. **Chiang Mai:** Payap. Chiangrai, en route from Ban Lang Lat to middle elevation of Doi Pacho (Doi Langka), *F. Iwatsuki et N. Fukuoka T3558* (AAU, BKF). **Udawn:** Loey, Phu Luang, *M. Tagawa et al. 1611* (BKF).

33. Ainsliaea yunnanensis Franch., J. Bot. (Morot) 2: 70. 1888. *Ainsliaea latifolia* (D. Don) Sch. Bip. f. *yunnanensis* (Franch.) Kitam., Acta Phytotax. Geobot. 41: 171. 1990. *Ainsliaea pteropoda* DC. var. *leiophylla* Franch., J. Bot. (Morot) 2: 69. 1888. TYPE: China. Yunnan: Che-tcho-tze, "supra Tapin-tze," 3 Oct. 1882, *J. M. Delavay 608* (type, NY!). Figure 43.

Ainsliaea scabrida Dunn, J. Linn. Soc., Bot. 35: 510. 1903. TYPE: China. Yunnan: Mi lê, in woods at Mengtze, *A. Henry 9851* (lectotype, designated here, MO!; duplicates, K not seen, K photo LP!).

Plants perennial, herbaceous, 0.25–0.70 m tall; stems erect, unbranched, leafy, lanuginose. Leaves basally clustered, rosulate, long-petiolate, petioles

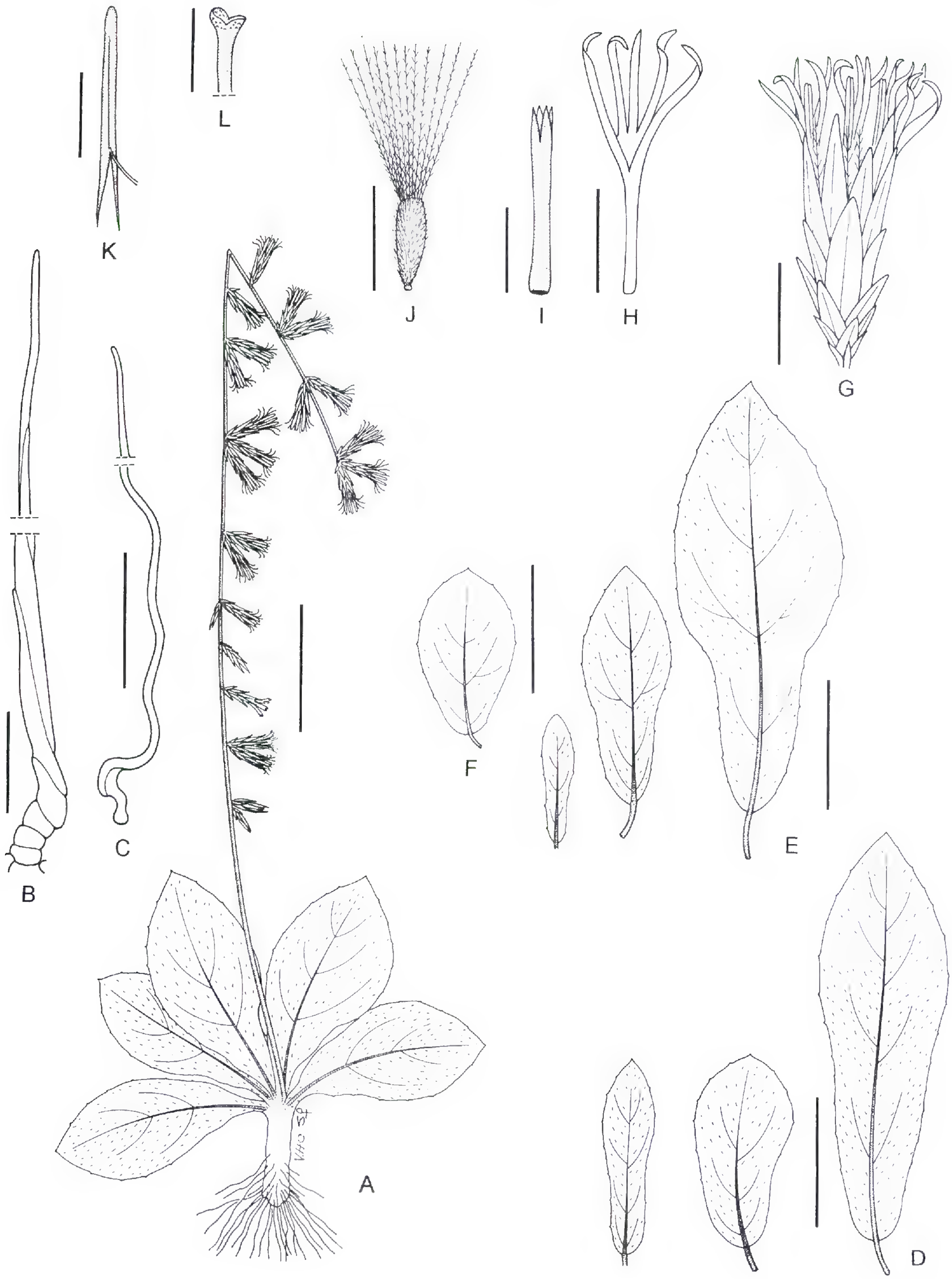


Figure 42. *Ainsliaea spicata* Vaniot. —A. Habit. —B, C. Foliar trichomes. —D. Leaves: cauline leaf and basal leaves (from left to right). —E. Leaves: cauline leaf and basal leaves (from left to right). —F. Basal leaf. —G. Capitulum. —H, K, L. Chasmogamous floret: H, corolla; K, stamen; L, style branches. —I, J. Cleistogamous floret: I, corolla; J, achene with pappus. Scale bars: A, D–F = 3 cm; B, C = 0.1 mm; G, H, J = 5 mm; I, K = 2 mm; L = 1 mm. Drawn from A, G–L, *Maire s.n.* (W7228); B, C, *Delavay 1921A* (P); D, *Henry 9294A* (NY); E, *Kapoort 75623* (MO); F, *Handel-Mazzetti 864* (W).

Figure 43. *Ainsliaea yunnanensis* Franch. —A. Habit. —B, C. Foliar trichomes. —D. Capitulum. —E. Phyllaries. —F–I. Chasmogamous floret: F, corolla; G, stamen; H, style branches; I, achene with pappus; J, style. Scale bars: A = 3 cm; B = 0.1 mm; C = 0.02 mm; D–F, I, J = 5 mm; G = 2 mm; H = 1 mm. Drawn from A, D–J, *Cavalerie 22* (S); B, C, the type *Delavay 608* (NY).

wingless, 2.5–4.5 cm; leaf blades coriaceous, ovate, 2.5–5.5 × 1.2–3 cm, acute at the apex, abruptly contracted at the base, then cuneately narrowed into the petiole, margins denticulate; roughly strigose on both surfaces, with long T-shaped trichomes and few glandular trichomes uniseriate; palmati-pinnate, 3-nerved. Capitula sessile, arranged in a stachyoid inflorescence; involucre 5-seriate, cylindrical, 13–15 × 3–7 mm; phyllaries subcoriaceous, purple apically, glabrous; outer phyllaries ovate, acute, ca. 6 × 2.5 mm; inner phyllaries lanceolate, acute, midvein dark, ca. 11 × 2.5 mm. Florets 3, hermaphrodite, chasmogamous; corolla pink, ligulate 14–15 mm, lobes 5–7 mm (deeper slit 7–8 mm), tube 7–7.5 mm; anthers 8–9 mm, apical appendage rounded, ca. 1 mm, sagittate; tails ca. 2.5 mm, smooth to slightly pilose; style branches shortly bilobate, truncate, ca. 0.3 mm. Achenes slightly oblong to obovate, 3–4 mm, 10-nerved, densely pilose; pappus bristles 32 to 37, ca. 7.5 mm.

Distribution and phenology. Southern China (Yunnan), collected on mountain slopes at elevations between 1600 and 3640 m; flowering in March, May, September, October, and December.

Discussion. Two syntypes from Yunnan are mentioned in the protologue of *Ainsliaea scabrida*: "mountain slopes at 6000 ft. near Mengtze, *Hancoock 8*" and "in woods at Mengtze, *A. Henry 9851*." I selected as lectotype the collection "*A. Henry 9851*" kept at MO, which is better preserved.

Relationships. *Ainsliaea yunnanensis* resembles *A. parvifolia* in its leaves. Both species have long-petiolate leaves, with leaf blades that are coriaceous or subcoriaceous and cuneate at the base. *Ainsliaea yunnanensis* may be distinguished by its foliar T-shaped trichomes (vs. straight trichomes), achenes pilose (vs. pilose above), and its larger involucres, 13–15 mm (vs. 8–10 mm).

Representative specimens examined. CHINA. **Yunnan:** Yunnan Sen, *M. M. Bodinier & F. Ducloux 8* (MO); Kunming, *P. I. Chiou 60097* (CM); Yünnanfu, *J. Cavalerie 22* (S), Yunnan, *J. M. Delavay in 1895* (UC, W); mountain slopes near Mengtze, *W. Hancock 8* (paratype of *A. scabrida*, K photo LP); Yunnan Sen, *E. E. Maire 919* (C, UC, W); Yunnan Kin-tchong-chan, *E. E. Maire 2529* (NY, UC); Yunnan, *E. E. Maire 3947* (UC); Kin-tchoug-chan, *E. E. Maire s.n.* (W7224); W of Talifu, Mekong watershed, rd. to "Youngchang" [Shuangjiang] and Tengyueh, Mt. above Hoa Chao, *J. F. Rock 6937* (US); "Berg über Podjo w.v. Tunghai," *H. v. Wissmann 184* (W); "Zw. Oschan & Podjo w.v. Tunghai," *H. v. Wissmann 230* (W); Molangpo bei "Yuendjiang" [Shuangjiang], *H. v. Wissmann 411* (W). **Unknown:** Hu-Chan-men, *J. M. Delavay in Oct. 1889* (US, W); s. loc., *K. Hou 74236* (MO).

C. *AINSLIAEA* SECT. *AGGREGATAE* BEAUVERD

Ainsliaea sect. **Aggregatae** Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 376. 1909. TYPE: *Ainsliaea acerifolia* Sch. Bip., lectotype, designated here.

Ainsliaea sect. *Aggregatae* was circumscribed by Beauverd (1909: 376) as containing 14 species (see Taxonomic History), no species name being designated by Beauverd as the type. *Ainsliaea acerifolia*, which is in accordance with the protologue, is here designated as lectotype of *Ainsliaea* sect. *Aggregatae* Beauverd.

Perennial herbs; stems erect, unbranched. Leaves clustered near the median part of the stem (rarely rosulate). Florets usually 3, rarely 2; anthers with apical appendages truncate or rounded, sometimes apiculate or emarginate, and tails usually shortly pilose, sometimes smooth to slightly pilose or shortly pilose.

Fourteen species from eastern China (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, rarely Sichuan and Yunnan, and Zhejiang), Korea, Taiwan, and Japan.

Key to the species of Ainsliaea *section* Aggregatae *in Asia*

1a. Leaf blades linear to elliptic or elliptic (0.3–0.9 cm wide).
  2a. Blades linear to elliptic, apex acute, 1-nerved.
    3a. Leaf margins with 4 to 8 teeth on each side . . . . . 9. *A. linearis*
    3b. Leaf margins with 1 or 2 subterminal teeth on each side . . . . . 14. *A. walkeri*
  2b. Blades lanceolate, 5–13 mm wide, apex acuminate, 3-nerved . . . . . 13. *A. trinervis*
1b. Leaf blades lobed, parted, or entire (more than 0.9 cm wide).
  4a. Anther appendages apiculate.
    5a. Leaves deeply 3-parted . . . . . 5. *A. dissecta*
    5b. Leaves lobed, but not 3-parted, with more than 3 lobes.
      6a. Blades palmately lobed, slightly cordate at the base . . . . . 1. *A. acerifolia*
      6b. Blades pinnately lobed, sagittate at the base . . . . . 4. *A. cordifolia*
  4b. Anther appendages rounded.
    7a. Leaves lobed.
      8a. Blades palmately lobed . . . . . 2. *A. apiculata*
      8b. Blades shallowly pinnately lobed . . . . . 7. *A. grossedentata*
    7b. Leaves not lobed.
      9a. Blades lanceolate, attenuate at the base . . . . . 11. *A. oblonga*

9b. Blades ovate, rounded or cordate at the base.
10a. Blades 2–3.5(7) cm long.
11a. Blades chartaceous, usually purplish green on lower surface . . . . . 6. *A. gracilis*
11b. Blades subcoriaceous, green on lower surface . . . . . 12. *A. parvifolia*
10b. Blades usually more than 4 cm long.
12a. Achenes glabrous or pilose above . . . . . 8. *A. kawakamii*
12b. Achenes pilose.
13a. Blades white-lanate on lower surface (with long crisped trichomes) . . . . . 3. *A. apteroides*
13b. Blades green on lower surface (with long straight trichomes) . . . . . 10. *A. macroclinidioides*

1. Ainsliaea acerifolia Sch. Bip., Jahresber. Pollichia 18–19: 188. 1861. TYPE: Japan. *H. Burger & H. Zollinger 272* (holotype, P!). Figure 44.

Plants perennial, herbaceous, 0.25–0.80 m tall; stems erect, unbranched, loosely strigose. Leaves 4 to 7, clustered near the middle of stems, falsely verticillate, long-petiolate; petioles wingless, flat, slightly shorter than the blades, slightly strigose; leaf blades chartaceous, cordate at the base, palmatilobed, lobes acuminate, mucronate-toothed, with terminal segments 2- or 3-dentate-lobed; dark green above and pallid below; subglabrous on both surfaces, veins somewhat more densely pubescent, with long straight trichomes, 0.6–1.5 mm long; palmativeined. Capitula subsessile, arranged in a stachyoid inflorescence; peduncles 1–2 mm, with many minute bracts and the upper transitioning to involucral bracts, involucre 7- or 8-seriate, cylindrical, 11–15 × 4–5 mm; phyllaries subcoriaceous, glabrous, or apically slightly strigose; outer phyllaries very short, ovate, the apices obtuse, 1.5–2 × 1–1.5 mm; inner phyllaries oblong, the apices subobtuse, 3- to 5-nerved, 10–11 × ca. 2 mm. Florets 3, hermaphrodite, chasmogamous, corollas white, ligulate, ca. 15 mm, lobes ca. 8 mm (deeper slit ca. 9 mm long), tube ca. 6 mm; anthers 7–7.5 mm, apical appendages apiculate, 0.4–0.5 mm, sagittate; tails 1.7–2 mm, shortly pilose; style branches short-lobed, rounded, ca. 0.7 mm. Achenes oblong, usually flattened, 3.5–8 mm, 10-nerved, glabrous; pappus bristles 36 to 50, 8–10 mm.

Distribution. Japan (Honshu) and South Korea; variety *subapoda* also extends to Liaoning, China (Fig. A).

Relationships. *Ainsliaea acerifolia* most resembles *A. lancangensis*. Both have leaves arranged at the median part of the stem, with blades palmately lobed and apiculate anther appendages. *Ainsliaea acerifolia* differs from *A. lancangensis* by its 3-flowered capitula. In contrast, the capitula of *A. lancangensis* are 1-flowered.

Key to varieties of Ainsliaea acerifolia *in Japan and South Korea*

1a. Leaf blades deeply 7- to 9(11)-lobed . . . 1a. var. *acerifolia*
1b. Leaf blades shallowly 5- to 7-lobed . . . 1b. var. *subapoda*

1a. Ainsliaea acerifolia Sch. Bip. var. **acerifolia**. Figure 44A–F.

Leaves deeply 7 to 9(11)-lobed, base cordate, blades 10–13 × 10–15 cm, petiole 6–9 cm; 7- to 9(11)-nerved.

Distribution and phenology. Japan (Kyushu), woods in mountains; flowering collection without month (Fig. 3A)

Vernacular names. Momiji-haguma (Kitamura, 1965), Momidzi-haguma (Iinuma, 1862; Masamune, 1934).

Representative specimens examined. JAPAN. **Locality unknown:** Ex Herb. Lugd. Batav., s. coll. (S, W). Kyushu. "In montib. Kund-sho-jan" [Kundjhojan], *C. J. Maximowicz in 1863* (M, MO, S, US, W).

1b. Ainsliaea acerifolia Sch. Bip. var. **subapoda** Nakai, Bot. Mag. (Tokyo) 30: 290. 1916. TYPE: Korea. Phying-an bor.: Nyag-suidong, s.d., *T. Ishidoya s.n.* (holotype, TI not seen; isotype, TI photo LP!). Figure 44G, H.

Leaves shallowly 5- to 7-lobed with broad lobes, cordate to subcordate at the base, blades 5–14 × 5.5–18 cm, petiole 4–12 cm; 5- to 7-nerved.

Distribution and phenology. China (Liaoning), South Korea, and Japan (Honshu). Commonly collected on humus slopes in *Abies* Mill., *Quercus* L., or *Chamaecyparis* Spach-*Cryptomeria* D. Don forest at elevations between (70/200)500 and 2500 m; flowering from August to October.

Discussion. Miquel (1866: 187) published his *Ainsliaea affinis* on "*Keiske lectum.*" Kitamura (1938) considered *A. affinis* Miq. as a variety of *A. acerifolia* and made the new combination *A. acerifolia* var. *affinis* (Miq.) Kitam. Even if I were able to find the type collection of *A. affinis* ("*Keiske lectum*") in G, L, or U, where the original herbarium of Miquel is probably deposited, the protologue of *A. affinis* agrees in all respects with *A. acerifolia* var. *subapoda*.

Vernacular names. Oku-Momiji-haguma (Kitamura, 1965; *Furuse in 1958, in 1961*, S), Momiji-

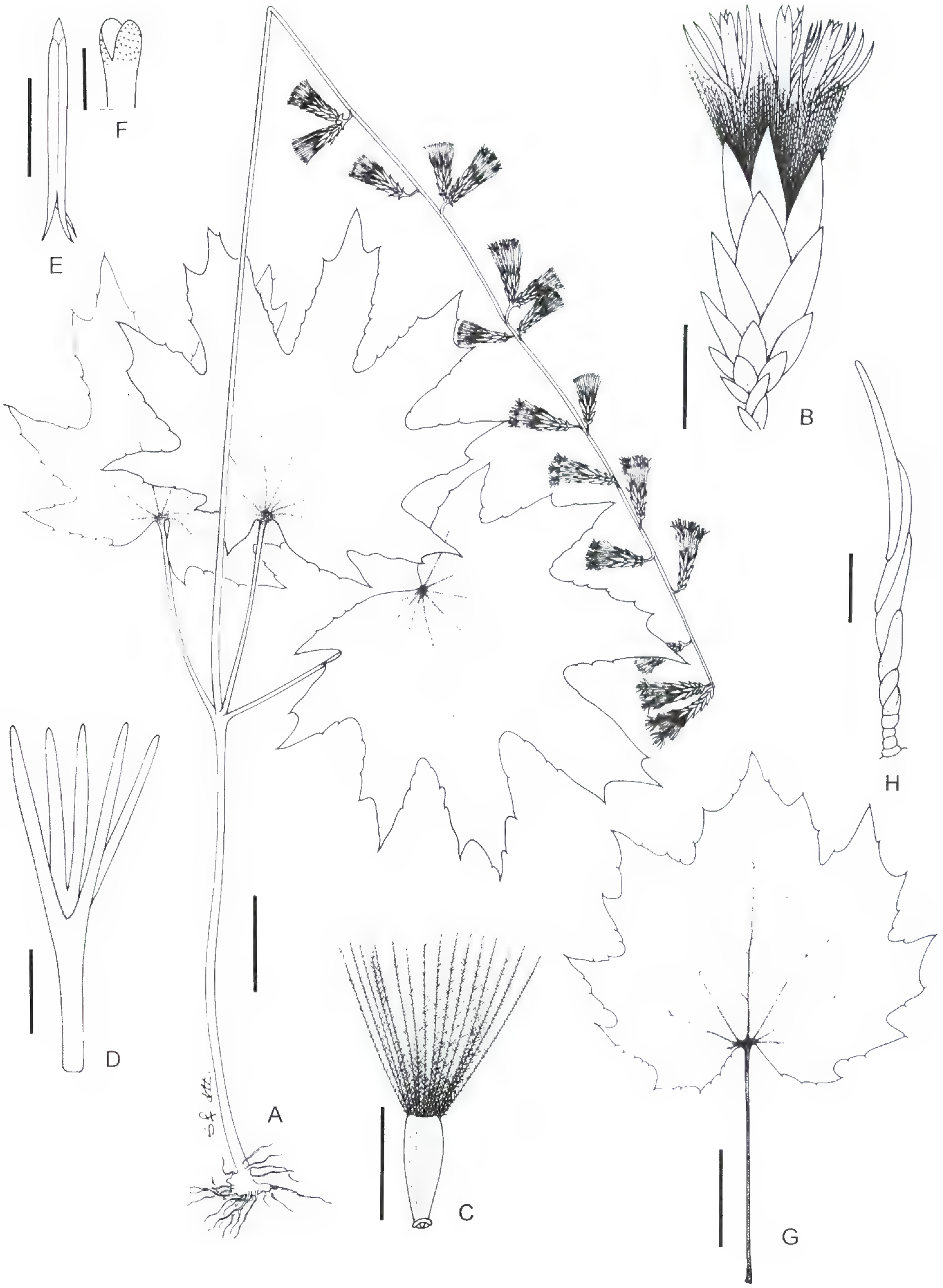


Figure 44. *Ainsliaea acerifolia* Sch. Bip. A–F. *A. acerifolia* var. *acerifolia*. —A. Habit. —B. Capitulum. —C–F. Chasmogamous floret: C, achene with pappus; D, corolla; E, stamen; F, style branches. G, H. *A. acerifolia* var. *subapoda* Nakai. —G. Leaf. —H. Foliar trichome. Scale bars: A = 3 cm; B = 5 mm; C = 5 mm; D, E = 3 mm; F = 1 mm; G = 3 cm; H = 0.1 mm. Drawn from A–F, *Maximowicz in 1863* (MO); G, *Boufford & Kato 23362* (MO); H, *Tagawa 557* (AAU).

haguma (*Suzuki 107*, UC), Oku-momizigasa (*Suzuki 145*, UC), Momiji-komori (*Hiroe 1167*, UC).

Representative specimens examined. CHINA. **Liaoning:** *S. C. Cui 314* (PE). JAPAN. Honshu. **Aichi:** Mikawa, Orimoto-tooge, Kami-tsugu-mura, Kita-shitara-gun, 28 Sep. 1955, *M. Furuse s.n.* (S). **Chiba:** Shimoosa, Mt. Ogura, Nikko, *M. Hiroe 1167* (UC). **Fukushima:** Iwaki, Mt. Ohtakine, Takine-machi, Tamura-gun, 7 July 1956, *M. Furuse s.n.* (S); Nishi-shirakawa-gun, Kashi-yama, *K. Saito et al. in 1975* (MO). **Gunma:** Aizawa foot of Mt. Arafuna, Nishimoku-mura, Shimonita-machi, Kita-kamra-gun, 3 Sep. 1956, *M. Furuse s.n.* (S, UC); Mt. Myogi, *E. Hulten in 1961* (S); Kodzuke Mt. Akazawa, Agatsuma-gun, *S. Kobayashi 12921* (S); Kodzuke, Agatsuma-gun, Shima hot spring, *S. Kobayashi 15188* (S); Kodzuke, S slope of Mt. Akazawa, Sawada-mura, Agatsuma-gun, *M. Mizushima 12738* (S); Kodzuke, Mt. Arafune, Kanra-gun, *T. Satomi 14467* (S). **Ibaraki:** Hitachi, Mt. Tsukuba, Tsukuba-gun, *S. Kobayashi 13627* (S). **Ishikawa:** Hasadani to Koba, Komatsu-shi, *M. & T. Takeuchi 14* (CM). **Kyoto:** Yamasiro, Mt. Hiei-zan, *M. Tagawa 557* (AAU, C). **Miyagi:** Mt. Fubo-san, Namari-zawa, *D. E. Boufford & E. W. Wood 19871* (CM); Sendai, *U. Faurie 5451* (W), *U. Faurie 6740* (MO, UC); Marumori-cho, Abukuma Mtns., Kosai Pass, *J. Iketsu et al. 1099* (CM); Sendai, Saba-Hill, *Jisiba in 1927* (W 8655), *Jisiba in 1926* (S, UC); Aone-onsen, Kawasaki-cho, Shibata-gun, *G. Murata & H. Koyama 41255* (C, CM); Rikuzen, Mt. Funagata, *K. Shoma & M. Takahashi in 1974* (MO); Rikuzen, Mt. Kurikoma, *S. Sugaya et al. in 1952* (CM, MO); Shichigashuku-machi, E side of Mt. Ryuugadake, *K. Takaata & H. Kobayashi 575* (MO); Sendai-shi, Aoba-yama, *H. Takahashi 1838* (C, NY); Sendai, 9 Sep. 1923, s. coll. (W 4946), 17 Sep. 1923, s. coll. (W 3207). **Nagano:** Minami Alps, Kamiina-gun, Hase-mura, along the trail from Kitazawa-toge to the summit of Mt. Futago, *D. E. Boufford & T. Shimizu 23468* (MO); Shinano, Minami-saku-gun, Kita-maki-mura, side of Matsubara-Lake, 25 Sep. 1958, *M. Furuse s.n.* (S); Shinano Koshibu, Ohjika-mura, Shimo-ina-gun, 25 Aug. 1959, *M. Furuse s.n.* (S); Shinano, Mt. Eboshi to Mt. Toyokuchi, Shimoinagun, *G. Murata & T. Shimizu 1530* (S); Shizumo, Gov. Forest, Nagiso-cho, Nishichikumagun, *G. Murata et al. 150* (BKF); Hacchozaka to Jumojitoge, Minamisaku-gun, *T. Shimizu 13558* (S). **Nara:** Mt. Wasamata-yama, in the Mtns. Oominesan, Kamikitayama-mura, Yoshino-gun, *G. Murata 45392* (CM); NW slope of Mt. Wasamata, Kami-kitayama-mura, Yoshino-gun, *S. Tsugaru et al. 7348* (CM, MO). **Niigata:** Echigo, Kiyotsu-kyo, Nakauonuma-gun, *S. Kobayashi 12864, 14195* (S). **Saitama:** Mt. Mitsumine, *S. Suzuki 145* (UC). **Shiga:** Oomi, Sakata-gun, Mt. Ibuki, Ibuki-mura, 22 Sep. 1961, *M. Furuse s.n.* (S). **Tochigi:** Shioya-gun, Fujiwara-cho, Mt. Kogen N of Kinugawa Hot Spring off rte. 119, *D. E. Boufford & M. Kato 23362* (MO). **Tokyo:** Musashi, Mt. Takawo, Asakawa-machi, Minami-tama-gun, Tokyo-to, 24 Oct. 1958, *M. Furuse s.n.* (S); "Musasi," Takoasan, 17 Oct. 1907, s. coll. (S). **Toyama:** W Mtn. (Nishi Yama), Sukubo, Yatsuo Town, 20 km NE of Toyama, *L. A. Charette 1897* (MO, US); Toyama-Ken, Nakaniikawa-gun, Kamiichi-machi, Takamine-yama, *J. Jutila & H. Fujino 751* (MO); Yatsuo Village, 17 km SW of Toyama, *S. Kirino 392* (M, MO); Tateyama Nat. Park, Bunazaka, ca. 2.5 km from Bijodaira, Mt. Tateyama, *F. H. Utech 98-415* (CM). **Yamagata:** Higashine-shi, Yanagisawa-goya to Mt. Funagatayama, *K. Inoue et al. 1760* (NY); Yamagata-shi, Kabuto-iwa (the interior of Yamadera), *H. Ohashi et al. 708054* (MO). **Yamanashi:** Kai, Higashi-yamanashi-gun, Mt. Kemtoku, Mitomi-mura, 14 Sep. 1957, *M. Furuse s.n.* (S); rd. from Yashajin-pass to Minami-omuro lodge, Ashiyasu-mura, Nakakoma-gun, *E. Miki et al. 56* (MO); Mt. Hoowoo, *S. Suzuki 107* (UC). **Unknown:** Oginohama, *U. Faurie 6356* (MO); Tidesan, *U. Faurie 1918* (WU); Togakushi, *U. Faurie 1920* (WU); Raminatake, *Raince & Kessliz in 1886* (W8047); Chitsan, Keisyonando, Chosen Nippon, *K. Uno 23338* (NY). SOUTH KOREA. Kwang Nung, Dan Pung Chui, *B. C. Chung in 1960* (MO); Kwangnung, Kyonggi-Do, *I. C. Chung 7080* (S); "Plantae Coreaneae," *U. Faurie 409* (UC); Kwang Nung, Dan-pung-chui, Mt. Kwan Aek, *I. K. Lee in 1957* (MO); Kyonggi, Kwang Nung, *Y. N. Lee & Y. S. Lee 709* (US); Paegyangsan, *R. Moran 4722* (UC, US).

2. Ainsliaea apiculata Sch. Bip., Jahresber. Pollichia 18–19: 188. 1861. TYPE: Japan. *H. Bürger & H. Zollinger 266* (lectotype, designated here, P!). Figure 45.

Plants perennial, herbaceous, 0.8–0.30 m tall; stems 1(2) erect, unbranched (occasionally branched at the base of the inflorescence), few leafy, loosely strigose. Leaves basally clustered, rosulate, long-petiolate; petioles wingless, twice as long as the blades, 1.5–6 cm, strigose; leaf blades chartaceous, usually palmately lobed; blackish green, slightly glassy above and pale opaque below, sparsely strigose on both sides, with long straight trichomes; cauline leaves few, much reduced, alternate, short-petiolate to sessile, ovate to ovate-lanceolate. Capitula subsessile, arranged in a stachyoid inflorescence; peduncules with many minute bracteoles, with upper bracts transitioning to involucre; involucre 5- or 6-seriate, narrowly obdeltate, (8)11–15 × ca. 3 mm; phyllaries papyraceous, glabrous; outer bracts ovate, apically acute, ca. 2 × 1 mm, inner bracts linear, apically acute, 3- to 5-nerved, ca. 10 × 1 mm. Florets 3, hermaphrodite, cleistogamous and chasmogamous (at different seasons and within a single plant), corollas white; *chasmogamous florets* with corolla ligulate, 9–11 mm, lobes 3–5 mm (deeper slit 4–6 mm), tube ca. 5 mm; anthers ca. 3 mm, apical appendages rounded, ca. 0.5 mm, sagittate; tails 1.2–1.5 mm, shortly pilose; style branches short, truncate, ca. 0.3–0.4 mm. Achenes oblong, ca. 2 mm long, short-pilose, 10-nerved; pappus bristles 34 to 36, ca. 6 mm; *cleistogamous florets* with corolla tubulose to pseudo-ligulate, 5-lobed, 5–6 mm, lobes 0.5 mm (deeper slit ca. 0.8 mm), tube 4–5 mm; anthers ca. 2 mm; style branches ca. 0.3 mm. Achenes ca. 5 mm; pappus bristles ca. 9 mm.

Distribution. Japan (Honshu, Kyushu), Taiwan, and South Korea (Fig. 3B).

Discussion. Schulz Bipontinus (1861) mentioned two syntypes in the protologue of *Ainsliaea apiculata*: "Japonia: Dr. *Bürger! et Zollinger! N.266 et ed. Göring n.217b.*" I selected as lectotype *Bürger & H.*

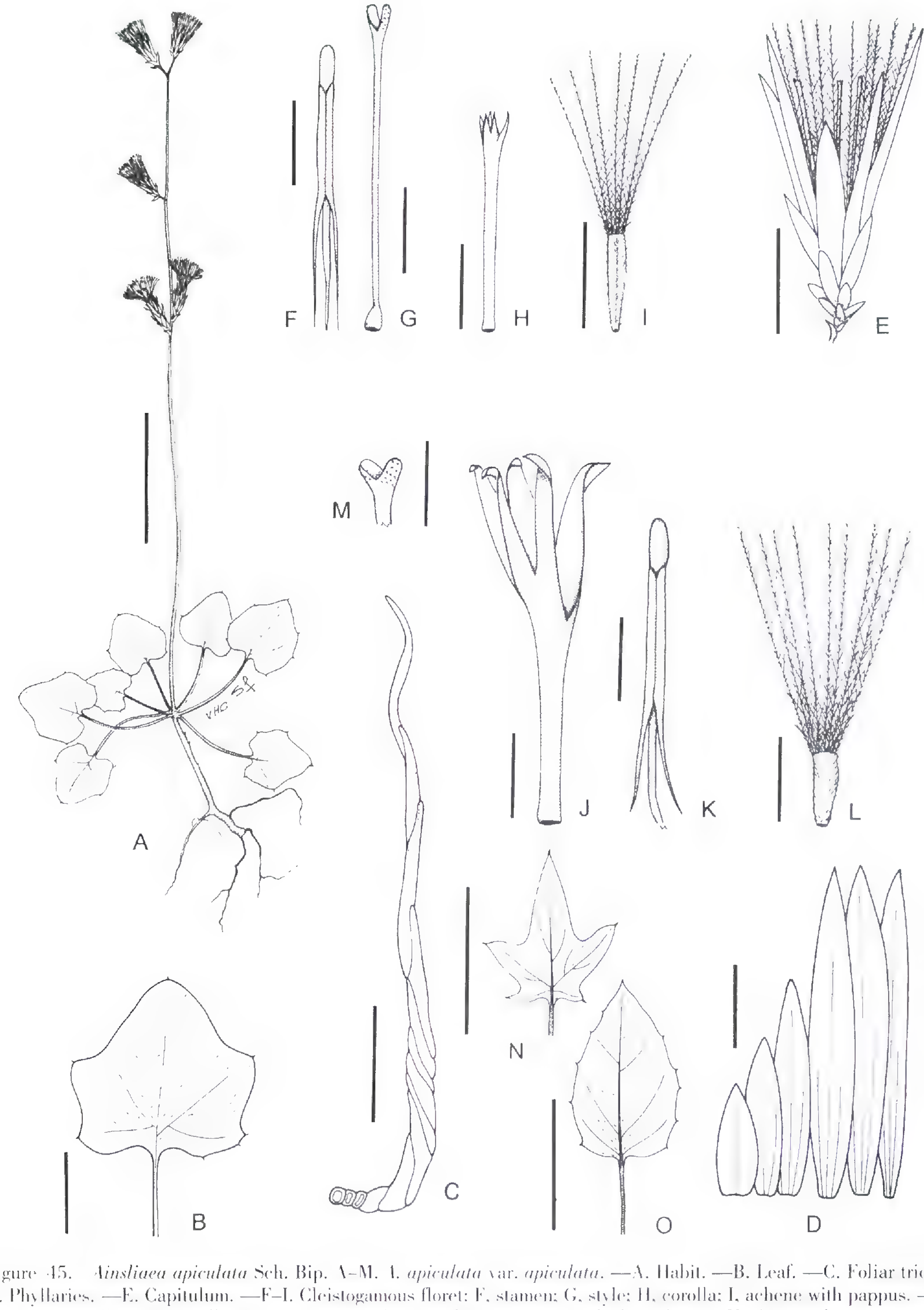


Figure 45. *Ainsliaea apiculata* Sch. Bip. A–M. *A. apiculata* var. *apiculata*. —A. Habit. —B. Leaf. —C. Foliar trichome. —D. Phyllaries. —E. Capitulum. —F–I. Cleistogamous floret: F, stamen; G, style; H, corolla; I, achene with pappus. —J–M. Chasmogamous floret: J, corolla; K, stamen; L, achene with pappus; M, style branches. —N. *A. apiculata* var. *acerifolia* Masam. Leaf. —O. *A. apiculata* var. *ovatifolia* Masam. Leaf. Scale bars: A = 2.5 cm; B = 1.5 cm; C = 0.2 mm; D, H, J, L = 2 mm; E, I = 5 mm; F = 0.5 mm; G, K, M = 1 mm; N = 3 cm; O = 2 cm. Drawn from A, D–M, *Furuse in 24 Oct. 1958* (S); B, C, *Furuse in 4 Nov. 1955* (S); N, the neotype *Muramatsu in 1935* (KYO); O, *Tashiro in 1918* (KYO).

Zollinger 266, kept at P, because I was unable to see the syntype *Göring 217b* deposited at P.

Miquel (1866: 187) established *Ainsliaea apiculata* var. *minor* because of its leaves 1.2 cm long and stems between 5 and 12 cm tall. Although I was unable to find the collection "*In m. Higosan, m. Nov fructiferam, Siebold.*" on which Miquel based *A. apiculata* var. *minor*, these characters are within the range of variation for *A. apiculata*.

Masamune (1934) described five varieties of *Ainsliaea apiculata* from Japan. Kyushu (Yakushima), i.e., *A. apiculata* var. *scapifolia*, *A. apiculata* var. *multiscapa*, *A. apiculata* var. *rotundifolia*, *A. apiculata* var. *acerifolia*, and *A. apiculata* var. *ovatifolia*, based on "Ipse, 7 June 1928," "Ipse, Kosugidani, ca. 600 m, Aug. 1928," "Ipse, July 25 1924," "Ipse, July 29, 1924," and "Ipse, Kosugidani, 1928," respectively. I have been unable to locate these collections, including at the herbaria where they should be located (KANA, TAI, TI). Although it is difficult to ascertain the identities of these taxa without type material, according to the protologues, *A. apiculata* var. *scapifolia*, *A. apiculata* var. *multiscapa*, and *A. apiculata* var. *rotundifolia* are based on characters that are within the range of variation for *A. apiculata* (leafy stems with obovate to lanceolate leaves; branched stems with three to five palmate to dentate leaves, and rounded to cordate leaves, respectively). However, the characters mentioned by Masamune for *A. apiculata* var. *acerifolia* and *A. apiculata* var. *ovatifolia* separate them as distinct taxa. Consequently, *A. apiculata* is recognized in this treatment as a complex of three varieties growing sympatrically, which are probably separated ecologically or by their mode of reproduction (only cleistogamous florets have been observed in varieties *acerifolia* and *ovatifolia*).

Key to the varieties of Ainsliaea apiculata *in Japan, Taiwan, and South Korea*

1a. Leaf blade entire or shallowly 5-lobed.
  2a. Leaf blade subrotundate or ovate, 5-angled or shallowly 5-lobed with terminal lobe often elongate, slightly cordate at the base; blades palmativeined . . . . . 2a. var. *apiculata*
  2b. Leaves ovate, acute, rounded at the base, mucronate-toothed on margins; blades palmati-pinnate . . . . . 2c. var. *ovatifolia*
1b. Leaf blade deeply lobed with elongated apical lobe . . . . . 2b. var. *acerifolia*

2a. Ainsliaea apiculata Sch. Bip. var. **apiculata**. Figure 45A–M.

Leaf blades subrotundate or ovate, 5-angled or shallowly 5-lobed, 1–3.5 × 1–3 cm, lobes obtuse, mucronulate, terminal lobe often elongate. Florets all cleistogamous, cleistogamous and chasmogamous, or all chasmogamous.

Distribution and phenology. Japan (Honshu, Shikoku (Masamune, 1934), Kyushu) and South Korea. It was collected on hills and lower slopes in deciduous forest and *Cryptomeria japonica* (Thunb. ex L. f.) D. Don forest, at elevations between 140 and 1000 m; flowering from September to December.

Vernacular names. Kikkô-haguma (*Jisiba in 1927*, W; *Suzuki 93*, UC; Masamune, 1934), Maruba-kikkô-haguma (Masamune, 1934), Tagyô-kikkô-haguma (Masamune, 1934), and Tôzaki-kikkô-haguma, (Masamune, 1934), Sashi haguma (*Tanaka in 1872*, W).

Representative specimens examined. JAPAN. Honshu. **Aichi:** Mikawa, Kita-shitara-gun, Nagura-mura, Oo-nagura, along Kamsa R., 27 Oct. 1957, *M. Furuse s.n.* (S). **Chiba:** Mt. Kiyosumi, *S. Suzuki 141* (UC). **Gumma:** Kodzuke, Mt. Myogi, Kanra-gun, *S. Satomi 15301* (S). **Hiroshima:** Miyajima Isl., *M. Hiroe 12044* (UC). **Hyogo:** "Harima" [Arima], *S. Arimoto in 1913* (MO 3935593); Arima, Mt. Sepiko, Shikatani-mura, Shikama-gun, 24 Sep. 1960, *M. Furuse s.n.* (S). **Ibaraki:** Hitachi, Mt. Tsukuba, Tsukuba-machi, Tsukuba-gun, *S. Kobayashi 15169* (S). **Ishikawa:** Takatsuboriyama, Togi-machi, Hakui-gun, *H. Kanai 655* (BKF, CANB, M, NY, UC); Sagami, Mt. Shiro-yama, Nakano-machi, Tsukui-gun, *S. Kobayashi 12982, 15175* (S). **Kanagawa:** Fujiyama, *U. Faurie 6611* (MO); Sagami, Mt. Kimtoki, halfway from forest Hakone, 30 Sep. 1961, *M. Furuse s.n.* (S); Tagata-gun, Nekko kami-kano-mura, 4 Nov. 1955, *M. Furuse s.n.* (S p.p., UC); Idzu, Isl. Oshima, *S. Kobayashi 15072* (S); Sagami, Mt. Komagatake, Hakone, *S. Kobayashi 15174* (S); "Izu," Mt. Amagi, Tagata-gun, *S. Kobayashi 16547* (S); Mt. Ooyama, *S. Suzuki 93* (UC). **Mie:** Tado Range, Tado Shrine, *N. Takaki in 1957* (CM). **Miyagi:** Kessennuma, *U. Faurie 6079* (MO); Sendai, 3 Feb 1927, *Jisiba s.n.* (W). **Tochigi:** Shiobara, *U. Faurie 1220* (MO). **Tokyo:** Tokyo-to, Musashi, Minami-tama-gun, Asakawa-machi, Mt. Takawo, 24 Oct. 1958, *M. Furuse s.n.* (S); Tokio, *s. coll. 823*, p.p. (C); Oum-shi, Nagafuchi, *H. T. Im & T. Kawahara 7815* (NY); Yokohama, *O. Kuntze 3280* (NY); Yokohama, *C. Maximowicz 13147* (M, MO, W); Yokohama, *Waura 1493* (NY, W); Musashi, Takoasan, 17 Oct. 1907, s. coll. (S); Musashi, Ogikubo?, 21 Sep. 1910, s .coll. (US). **Toyama:** Yatsuo Village, 19 km SW of Toyama city, on slopes of Amid Mtns., *S. Kirino 356* (MO). **Yamaguchi:** in the village Misyo, 3 mi. SW of Yokoyama vill., Iwakuni (25.6 mi. W of Hiroshima city) base of W slope of Castle Mtn. (Shiro–Yama) Nat. Forest, N bank of Nishiki R., *L. A. Charette 1525* (MO, S, UC, US). **Wakayama:** Kii, Mt. Koya, near Okunoin, *M. Tamura 9327* (S). Kyushu. **Kagoshima:** Oshumi, Mt. Takakuma, *Kawagoe in 1917* (US), *G. Masamune in 1922* (NY); Yakushima, Osumi Gunto, along railroad from Ambo to Kasugidani at 16 km, *R. Moran 5333*, p.p. (UC); Yakushima, *Z. Tashiro in 1918*, p.p. (KYO); Yakushima, Kasugidani, *G. Yamaguchi & T. Nagano in 1917*, p.p. (KYO). **Nagasaki:** E middle elev. of Mt. Ariake, Izuhara-cho, Shimoagata-gun, Tsushima Isl., *H. Koyama 2678* (BKF, US); Nagasaki, *C. Maximowicz in 1863* (MO, S). **Unknown:** Kyushu, Hakogaki, Kasusjagun, Fukuskaken, *K. Ichikawa 128* (C, S, US, W); Sanchi Ojihen, *Y. Tanaka 16* (W); Takehara, *Y. Watabiki in 1902* (NY); *Goring 217b* (paratype of *A. apiculata*, P not seen; P photo LP!); s. loc., *A. Bay s.n.* (PENN 810779). SOUTH KOREA. Cheju-do (Quelpaert Island), 21 May 1946, *s. coll. 8823* (S); "in silvis Mallaisan," *T. Taquet* 243 (W); "Hallaiken"?, *T. Taquet 1062* (C).

2b. Ainsliaea apiculata var. **acerifolia** Masam. Mem. Fac. Sci. Taihoku Imp. Univ. 11(4): 455. 1934. TYPE: Japan. s. loc., 17 Aug. 1935, *Herb. Muramatsu s.n.* (neotype, designated here, KYO!). Figure 45N.

Ainsliaea liukiuensis Beauverd, Bull. Soc. Bot. Genève, Sér. 2, 1: 382. 1909. TYPE: Japan. Yakushima (Liu kiu), July 1900, *U. Faurie 4066* (type, KYO!).

Ainsliaea secundiflora Hayata, J. Coll. Sci. Imp. Univ. Tokyo 30: 162. 1911. Syn. nov. *Ainsliaea macroclinidioides* var. *secundiflora* (Hayata) Kitam., J. Jap. Bot. 14: 307. 1938. TYPE: Japan. Koshun, Botanrosha, Dec. 1906, *Nakahara 922* (holotype, TI not seen; isotype, TI photo A!, LP!).

Leaves (1)2.5–3.5 × (1.1)2–3 cm, deeply lobed with apical lobe elongate. Florets cleistogamous.

Distribution and phenology. Japan (Kyushu and Honshu) and Taiwan; collected in forest in mossy places, at elevations between 1600 and 1800 m; flowering in February, March, September, and October.

Discussion. Because no authentic material of *Ainsliaea apiculata* var. *acerifolia* ("*Ipse*, July 29, 1924") has been found at KANA, TAI, or TI, where the original herbarium of Masamune is kept (see discussion in *A. apiculata*), the collection *Muramatsu in 1935* (KYO), which agrees in all respects with the protologue of *A. apiculata* var. *acerifolia*, has been chosen as neotype.

Relationships. *Ainsliaea secundiflora* Hayata is proposed as a new synonym of *A. apiculata* var. *acerifolia* because there is no diagnostic character that differentiates the former from the latter. Both have long-petiolate leaves that are palmately 5-lobed with an elongate terminal lobe and deeply cordate at the base.

Vernacular names. Momiziba-kikkô-haguma (Masamune, 1934; Hori et al., 1988), Ryūkyū-haguma (Kitamura, 1965).

Representative specimens examined. JAPAN. Honshu. **Ishikawa:** Sagami, Mt. Shiroyama, Nakano-machi, Tsukui-gun, *M. Mizushima 13199* (S). **Kanagawa:** Tagata-gun, Nekko kami-kano-mura, 4 Nov. 1955, *M. Furuse s.n.*, p.p. (S). **Tokyo:** Tokio, *s. coll. 823*, p.p. (C). Kyushu. **Kagoshima:** Yakushima, Yaku-cho, Hana-no-eigo, *D. E. Boufford & S. Mitsuta 20043* (CM, MO); Yakushima, Aug. 1900, *U. Faurie s.n.* (KYO); Yakushima, Ohsumi, Sep. 1921, *G. Koidzumi s.n.* (AAU, CANB, KYO p.p., MO, PENN); Yakushima, Mt. Miyanonradake, Sep. 1921, *G. Koidzumi s.n.* (KYO); Isl. Yakushima, *K. Shimizu & I. Kozima 33* (KYO); Yakushima, inter Anbo et Kosugi-dani, *M. Tagawa & Motozi 1921* (KYO); Yakushima, Kiusiu, 6 Aug. 1918, *Z. Tashiro s.n.*, p.p. (KYO); Yakushima, 9 Aug. 1918, *Z. Tashiro s.n.*, p.p. (KYO); Yakushima, Kosugidami, 26 July 1917, *G. Yamaguchi & T. Nagano s.n.*, p.p. (KYO); Yakushima, Mt. Miyanoura, 28 July 1917, *G. Yamaguchi & T. Nagano s.n.* (KYO). TAIWAN. Prov. Takao, Mt. Daijurin, *S. Kitamura in 1932* (KYO); Mt. Arisan, *S. Sasaki in 1919* (KYO); Funki, *T. Sata in 1932* (MICH); Taitô-chô, Asahi, *T. Tanaka 10392* (C, NY, UC); Ping tung, Ali, *H. N. Yang 2039* (CM).

2c. Ainsliaea apiculata var. **ovatifolia** Masam., Mem. Fac. Sci. Taihoku Univ.: 455. 1934. TYPE: Japan. Kyushu. Kagoshima: Rte. from Takatskagoya to Mt. Miyanoura, Yaku-cho, 1300–1700 m, 3 Aug. 1974, *G. Murata & H. Tabata 418* (neotype, designated here, KYO!). Figure 45O.

Leaves ovate, 1.5–22 × 1.2–1.8 cm, apex acute, rounded at the base, mucronate-toothed on margins. Florets cleistogamous.

Distribution and phenology. Japan (Kyushu, Yakushima); collected at elevations between 1300 and 1700 m, in mossy places; flowering in August and December.

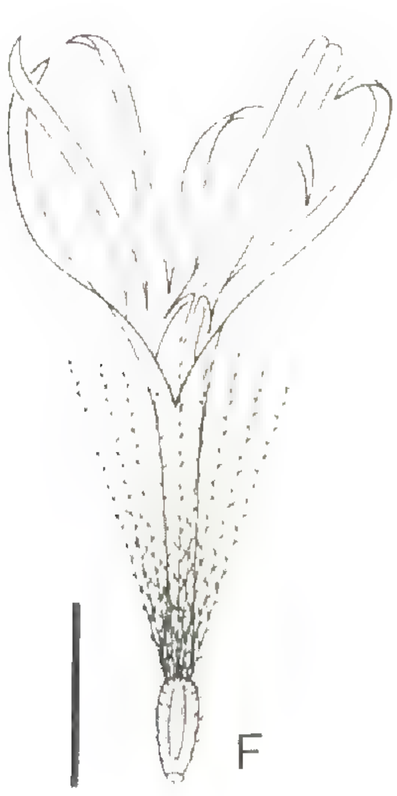
Discussion. Because no authentic material of *Ainsliaea apiculata* var. *ovatifolia* ("*Ipse*, Kosugidani, 1928") has been found at KANA, TAI, or TI, where the original herbarium of Masamune is kept (see discussion in *A. apiculata*), the collection *G. Murata & H. Tabata 418* (KYO), which agrees in all respects with the protologue of *A. apiculata* var. *ovatifolia*, has been chosen as the neotype.

Vernacular name. Tamagoba-kikkô-haguma (Masamune, 1934; Hori et al., 1988).

Representative specimens examined. JAPAN. Kyushu. **Kagoshima:** Yakushima, Ohsumi, *G. Koidzumi in 1921* (KYO p.p.); Yakushima, Ohsumi Gunto, along railroad from Ambo to Kosugidani at 16 km, *R. Moran 5333*, p.p. (UC); Yakushima, Kiusiu, 6 Aug. 1918, *Z. Tashiro s.n.*, p.p. (KYO).

3. Ainsliaea apteroides (C. C. Chang) Y. C. Tseng, Acta Phytotax. Sin. 31: 363. 1993. Basionym: *Ainsliaea pteropoda* var. *apteroides* C. C. Chang, Sinensia 4: 227. 1934. TYPE: China. Yunnan: Mi lêh district, mountain forest, *A. Henry 9907A* (holotype, K not seen; isotypes, K photo LP!, US!). Figure 46.

Plants perennial, herbaceous, 0.20–0.45 m tall; stems erect, unbranched, leafy, lanuginose. Leaves basally clustered, rosulate (occasionally clustered above the base), long-petiolate; petioles wingless, 4–5(9) cm, flat; leaf blades chartaceous, ovate, 5–8 × 3.5–5 cm, apex acute to acuminate (rarely obtuse), base slightly cordate to cordate, margins obscurely denticulate; upper surface sparsely lanate, lower surface white-lanate, with long crisped trichomes, and scattered long straight and short capitate glandular trichomes (subglabrate at maturity); palmati-pinnate, 3-nerved; cauline leaves alternate, shortly petiolate to sessile, similar to the base, gradually reduced upward to the base of the inflorescence, ovate to lanceolate or lanceolate, discolorous, upper surface lanuginose and lower surface densely white-tomentose. Capitula subsessile, to shortly pedunculate, arranged in a stachyoid in-



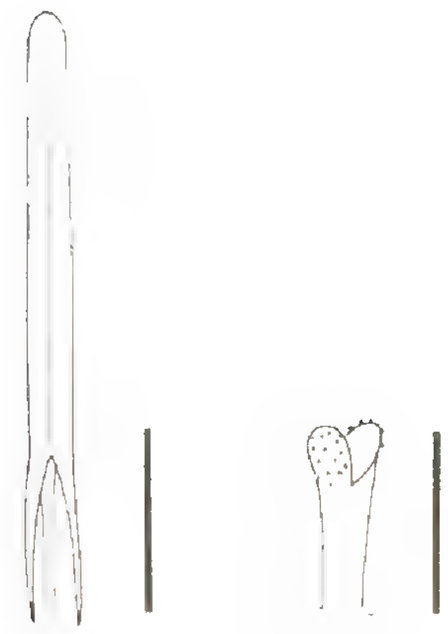


Figure 46. *Ainsliaea apteroides* (C. C. Chang) Y. C. Tseng. —A. Habit. —B, C. Foliar trichomes. —D. Phyllaries. —E. Capitulum. —F. Chasmogamous floret. —G. Stamen of chasmogamous floret. —H. Style branches of chasmogamous floret. —I. Cleistogamous floret. Scale bars: A = 3 cm; B, C = 0.1 mm; D = 3 mm; E = 5 mm; F, I = 5 mm; G, H = 2 mm. Drawn from A, D, E, the isotype *Henry 9907 A* (US); B, C, *Henry 9907* (NY); F–H, (W *7213*); I, *Biswas in 1935* (US).

florescence; peduncles bracteolate; involucre 5-seriate, cylindrical, 11–15 × 5–7 mm; phyllaries subcoriaceous, glabrous; outer phyllaries ovate, acute, 1.5–3 × 1–1.3 mm; inner phyllaries linear to lanceolate, acuminate, 3-nerved, 7–12 × 1–1.8 mm. Florets 3, hermaphrodite, chasmogamous and cleistogamous (at different seasons); *chasmogamous florets* with corollas ligulate, ca. 12 mm, lobes ca. 6 mm (deeper slit ca. 7 mm), tube ca. 5 mm; anthers ca. 5 mm, apical appendages truncate, ca. 1.2 mm, sagittate; tails ca. 2 mm, shortly pilose; style branches short, rounded, ca. 0.5 mm. Achenes linear-obovate, 5–6 mm long, 10-nerved, pilose; pappus bristles ca. 38, ca. 6 mm; *cleistogamous florets* with corollas tubulose to pseudoligulate, ca. 4 mm, shortly 5-lobed (deeper slit ca. 0.3 mm); anthers much reduced, ca. 1 mm; style branches ca. 0.6 mm. Achenes 5.5–6 mm; pappus bristles ca. 7 mm.

Distribution and phenology. Bhutan, China (Sichuan, Yunnan), and India in mountain forest, at ca. 1800 m; collected in flower in February.

Relationships. *Ainsliaea apteroides* resembles *A. bonatii* in habit and capitulescence. Both species have sessile capitula, rosulate leaves long-petiolate, with typically cordate leaf blades, and the lower blade surface white-tomentose. *Ainsliaea apteroides* has ovate leaf blades, acute to acuminate at the apex (vs. widely ovate to suborbicular leaf blades, obtuse to rounded at the apex), and petioles wingless (vs. broadly winged). *Ainsliaea apteroides* also resembles *A. aptera* in its ovate, acuminate leaf blades and petioles wingless, but *A. aptera* is quite distinct from *A. apteroides* by the leaf blades with dentate margins (vs. obscurely denticulate).

Representative specimens examined. BHUTAN. "Bootan," s. loc., s. coll. (W 7213). CHINA. **Yunnan:** Mengtze, *A. Henryi 9907* (MO, NY); Lunan Xian, *K. Hou 74327* (MO). **Sichuan:** Chiu-Lung-Hsien Terr. E of the Yalung River, *J. F. Rock 17427* (S, US, W). INDIA. Eastern Himalaya, *K. Biswas in 1935* (US).

4. **Ainsliaea cordifolia** Franch. & Sav., Enum. Pl. Jap. 2: 416. 1879. TYPE: Plate *sub.* "Teishô sô," in Sômoku-Dzusetsu, 16: 7. 1862 (lectotype, designated here). Japan. Honshu. Mie Prefecture: Prov. Kii, Kowadani, ravine Kowa, Owase-machi, Kitamuro-gun, 7 Oct. 1956, *M. Furuse s.n.* (epitype, designated here, S!; duplicate, UC!). Figure 47.

Ainsliaea maruoi Makino, Bot. Mag. 28(334): 290. 1914. TYPE: Japan. Honshu: Totomi, Aug. 1913. *S. Maruo s.n.* (holotype, MAK!).

Plants perennial, herbaceous, (0.20)0.30–0.45(0.70) m tall; stems erect, simple, rarely branched above, glabrous or loosely strigose. Leaves clustered at the median part of the stem or crowded above the base; longly petiolate, petioles wingless, flat, as long as the blade, 6–13.5 cm, sparsely strigose; leaf blades chartaceous, ovate to subreniform, (4)8–12(16) × (3.5)5–10.5(12.5) cm, sagittate at the base, obtuse to acute at the apex, pinnately lobed; subglabrous and green above, slightly paler and strigose with long straight trichomes below; palmati-pinnate, 3- to 5-nerved. Capitula subsessile, arranged in a stachyoid inflorescence; peduncles 2–3 mm, bracteolate, with upper bracts transitioning to involucre; involucre 7-seriate, 10–14 × 3–4 mm, narrowly obdeltate; phyllaries papyraceous, purple toward the margins above; outer phyllaries ovate, acute, ca. 3 × 1.5 mm, pilose at the apex; inner phyllaries narrowly oblong, acute, conspicuously 3- to 5-nerved, ca. 10 × 1 mm, glabrous. Florets 3, hermaphrodite, chasmogamous; corolla ligulate, ca. 15 mm, lobes 6–8 mm (deeper slit ca. 9 mm), tube ca. 6 mm; anthers 6–8 mm, apical appendages apiculate, ca. 1 mm, sagittate; tails ca. 1.5 mm, smooth to slightly pilose; style branches obtuse, ca. 0.7 mm. Achenes oblong, 4–6.5 mm, 10-nerved, glabrous; pappus bristles ca. 45, ca. 8 mm.

Distribution and phenology. Japan (Honshu, Shikoku), collected in shaded mountain forest (Fig. 4A); flowering in August, October, and November.

Discussion. The protologue of *Ainsliaea cordifolia* does not include any reference to the collector: "Hab. in montibus provinciarum insulae Nippon centralium, v.c. Kishiou, Yamato, Ise." The name of this species is accompanied by an illustration in Iinuma (1862, 16: 7), which is here selected as the lectotype. Because the illustration does not include dissections showing essential characters, I also propose the collection "*M. Furuse in 1956*" kept at S (duplicate, UC), which is in accordance with the protologue, as epitype of *A. cordifolia*.

Vernacular names. Teishô sô (Iinuma, 1862: Kitamura, 1965), Hiroha-teishoso (Makino, 1914).

Relationships. *Ainsliaea cordifolia* resembles *A. acerifolia* in its anthers and leaves. Both species have anthers with apiculate anther appendages and lobed leaves. *Ainsliaea cordifolia* is easily distinguished by its leaf blades pinnately lobed (vs. palmatilobed) and sagittate at the base (vs. cordate at the base).

Representative specimens examined. JAPAN. Honshu. **Chiba:** Awa, Awa-gun, Amatsu-machi, Mt. Kiyosumi on S side, 1 Nov. 1958, *M. Furuse s.n.* (S). **Tottori:** Totomi, Kasamisi-mura, 31 Oct. 1912, s. coll. (S). Shikoku. **Kagawa:** Shionoe-machi, Mt. Ohtaki-san, *M. Uchida in 1969* (CM). **Unknown:** Kiyosumi-san, Boshu, 28 Oct. 1894, s. coll. (US 248117); s. loc., July 1931, s. coll. (MAK 248107); s. loc.,

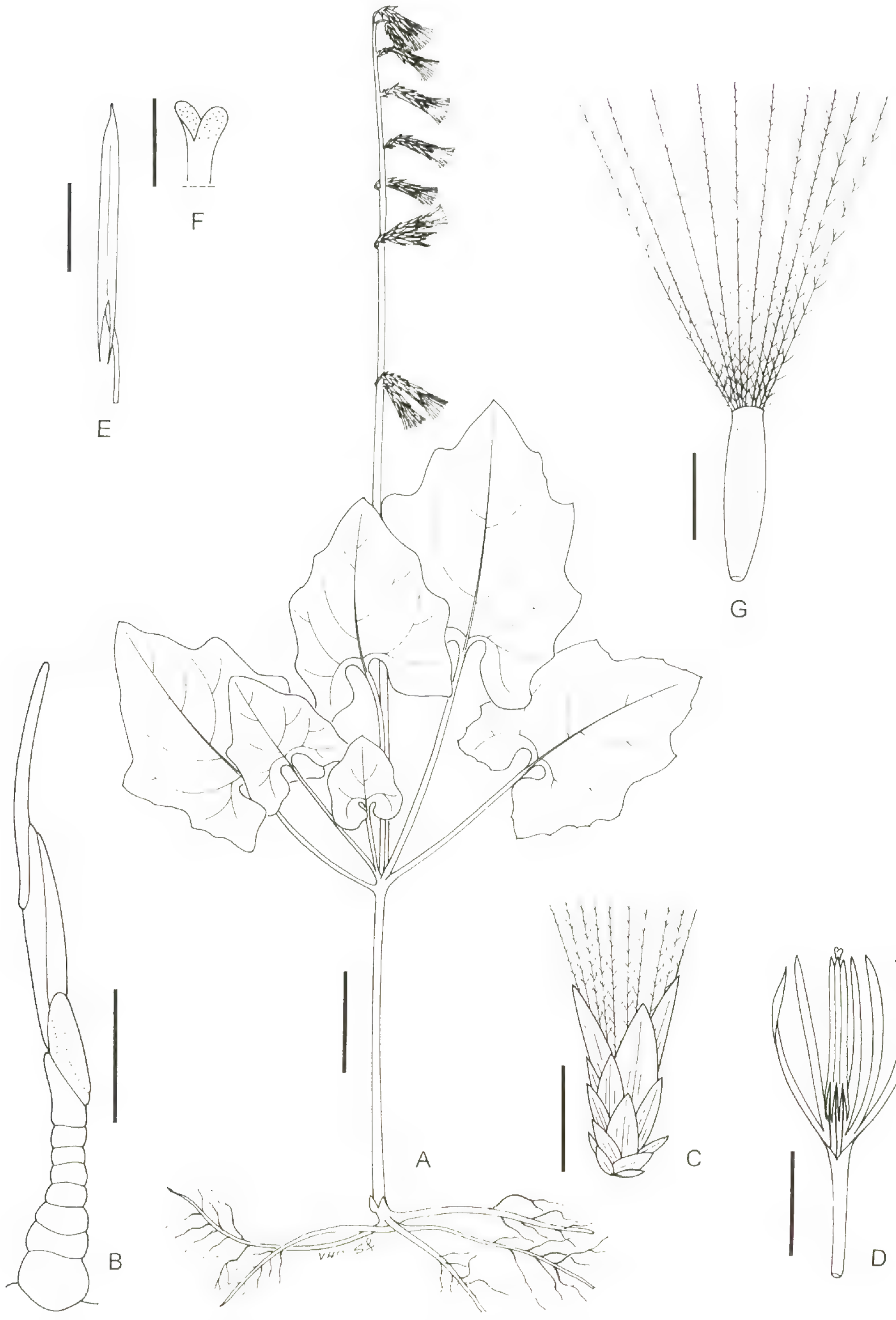


Figure 47. *Ainsliaea cordifolia* Franch. & Sav. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Chasmogamous floret without achene and pappus. —E. Stamen. —F. Style branches. —G. Achene with pappus. Scale bars: A = 3 cm; B = 0.1 mm; C, D = 5 mm; E, G = 2 mm; F = 1 mm. Drawn from A, C–G, Oct. 1894, s. coll. (US 248117); B, *Furuse in 1956* (UC).

year 1935, s. coll. (MAK 247863); s. loc., *N. Satomi in 1957* (MAK).

5. Ainsliaea dissecta Franch. & Sav., Enum. Pl. Jap. 2: 416. 1879. TYPE: Plate sub "Enshû-haguma" Sômoku-Dzusetsu, 16: 5. 1862 (lectotype, designated here). Japan. Honshu. Tottori Prefecture: Prov. Totomi, Mt. Hakko, Haibara-gun, 19 Oct. 1951, *J. Sugimoto 10912* (epitype, designated here, S!). Figure 48.

Plants perennial, herbaceous, 0.16–0.45 m tall; stems unbranched (occasionally branched above leaves, resulting in 3- or 4-flower stems), glabrous. Leaves crowded above the base, falsely verticillate, long-petiolate; petioles wingless, flat, about twice as long as the blades, 5–12(17) cm; leaf blades membranous, cordate at the base, palmately lobed to more commonly palmately 3-parted cordate, (2)4–5(7.5) × (2)4–5(8) cm, lateral segments deeply 2-lobed, terminal segment 3-lobed, lobes coarsely mucronate-toothed; subglabrous on both surfaces with long straight trichomes of 1–1.5 mm; palmativeined. Capitula subsessile, arranged in a stachyoid inflorescence; peduncles bracteolate, with upper bracts transitioning to involucre; involucre 7- or 8-seriate, cylindrical, 8–13 × 3–4 mm; phyllaries papyraceous, purpurescent toward the tip, glabrous; outer phyllaries ovate, acute, 1–1.5 × ca. 1 mm; inner phyllaries linear, acuminate, conspicuously 3- to 5-nerved, ca. 11 × 0.7 mm. Florets 3, hermaphrodite, chasmogamous; corolla ligulate, ca. 12.5 mm, lobes 6–6.5 mm (deeper slit ca. 7 mm), tube ca. 5 mm; anthers 5.5–7 mm, apical appendages apiculate, ca. 0.8 mm, sagittate; tails smooth to slightly pilose, 1.5–2 mm; style branches obtuse to acute, ca. 1 mm. Achenes oblong, flattened, 6.5–11 mm, 9-nerved, glabrous; pappus bristles 30 to 33, 8–12 mm.

Distribution and phenology. Japan (Honshu), collected in mountainous woods between 300 and 680 m (Fig. 4A); flowering in September and October, fruiting in November.

Discussion. The protologue of *Ainsliaea dissecta* does not include any reference to the collector: "Hab. in montibus editissimis, v. c. Fudsi yama, teste Inouma." The name of this species is accompanied by an illustration in Iinuma (1862, 16: 5), which is here selected as the lectotype. Because the illustration does not include dissections showing essential characters, I also propose the collection *J. Sugimoto 10912*, kept at S, which is in accordance with the protologue, as epitype of *A. dissecta*.

Vernacular names. Ensyu-haguma (*M. Furuse in 1955*, MO, S, UC), Enshû-haguma (Iinuma, 1862; Kitamura, 1965).

Relationships. *Ainsliaea dissecta* is easily distinguished from other species of the sect. *Aggregatae* with apiculate anther appendages (*A. acerifolia* and *A. cordifolia*) by its leaf blades that are palmately 3- to 5-divided.

Representative specimens examined. JAPAN. Honshu. **Aichi:** Prov. Mikawa, Mt. Hooraiji, Hooraiji-machi, Minami-shitara-gun, 28 Oct. 1957, *M. Furuse s.n.* (S); Mt. Danto, Oh-nagura, Nagura-mura, Kita-shitara-gun, 29 Sep. 1955, *M. Furuse s.n.* (S, UC); Mt. Horaijisan, Horai-cho, Minamishitara-gun, *G. Murata 41060* (CM). **Shizuoka:** Miyakoda, N of Hamamatsu, *F. Konta & M. Wakabayashi 357* (AAU, BKF, CM, MO, NY). **Tottori:** Prov. Totomi, Awagatake, s. coll. (S).

6. Ainsliaea gracilis Franch., J. Bot. (Morot) 8: 297. 1894. TYPE: China. Sichuan: Su-tchuen oriental, Tchen-keou-tin, *R. P. Farges 1034A* (type, P!). Figure 49.

Plants perennial, herbaceous, 0.20–0.45 m tall; stems erect, unbranched (rarely branched above), glabrous. Leaves clustered above the base, falsely verticillate, long-petiolate; petioles wingless, flat, subglabrous, 2–3.2 cm; leaf blades chartaceous, ovate or ovate to lanceolate, 2–3.5(7) × 1.9–3 cm, base slightly cordate or rounded to truncate, apex acuminate, margins obscurely dentate; discolorous, upper surface green, subglabrous, and lower surface purplish green, and loosely strigose with long straight trichomes. Capitula sessile to shortly pedunculate, arranged in a stachyoid inflorescence; peduncles bracteolate; involucre 5- or 6-seriate, cylindrical, ca. 10 × 3 mm; phyllaries papyraceous, glabrous; outer phyllaries ovate, acute, ca. 1.5 × 1 mm; inner phyllaries linear to lanceolate, acute to acuminate, 3- to 5-nerved, ca. 10 × 1.2 mm. Florets 3, hermaphrodite, chasmogamous and cleistogamous (usually in single plant), corollas white; *chasmogamous florets* with corolla ligulate, ca. 9 mm, lobes ca. 3 mm (deeper slit ca. 4.5 mm), tube ca. 4.5 mm; anthers ca. 4 mm, apical appendages rounded, ca. 0.5 mm, sagittate; tails ca. 1.5 mm, shortly pilose; style branches shortly bilobate, ca. 0.3 mm. Achenes linear to oblong, 2–4 mm, 10-nerved, glabrous or more commonly pilose above; pappus bristles ca. 37, ca. 5.5 mm; *cleistogamous florets* with corolla tubulose to pseudoligulate, ca. 5 mm, 5-lobed, lobes ca. 1.5 mm (deeper slit ca. 2 mm); anthers ca. 2 mm; style branches ca. 0.5 mm. Achenes 5 mm; pappus bristles ca. 8 mm.

Distribution and phenology. Central China (Guangdong, Guangxi, Guizhou, Hubei, Hunan, Sichuan). It was collected under shrubs on shaded banks, at elevations between 600 and 1500 m (Fig. 4B); flowering in August and October, fruiting in December.

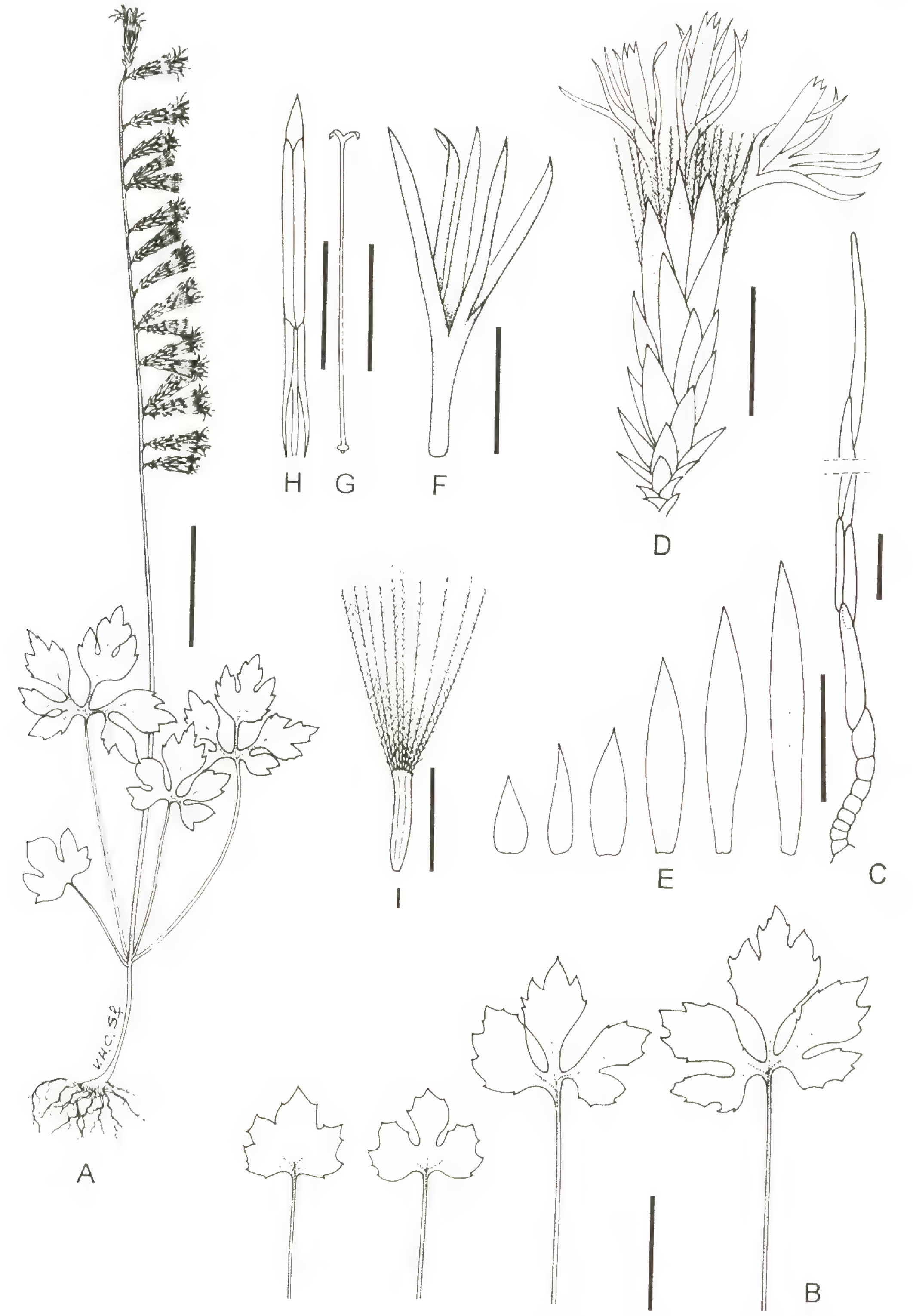
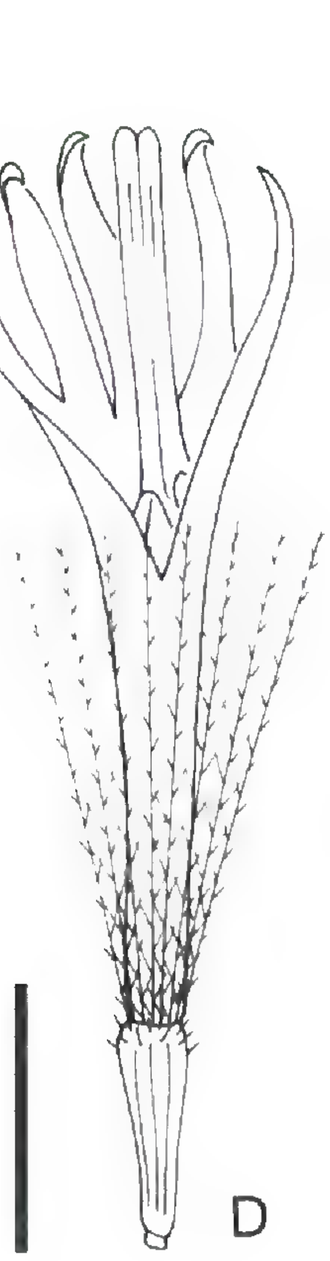


Figure 48. *Ainsliaea dissecta* Franch. & Sav. —A. Habit. B. Leaves from a single plant. —C. Foliar trichome. —D. Capitulum. —E. Phyllaries. —F–I. Chasmogamous floret: F, corolla; G, style; H, stamen; I, achene with pappus. Scale bars: A, B = 3 cm; C = 0.1 mm; D–G, I = 5 mm; H = 2 mm. Drawn from A, C–I, *Furuse in 29 Sep. 1955* (UC); B, *Konta & Wakabayashi 357* (AAU).



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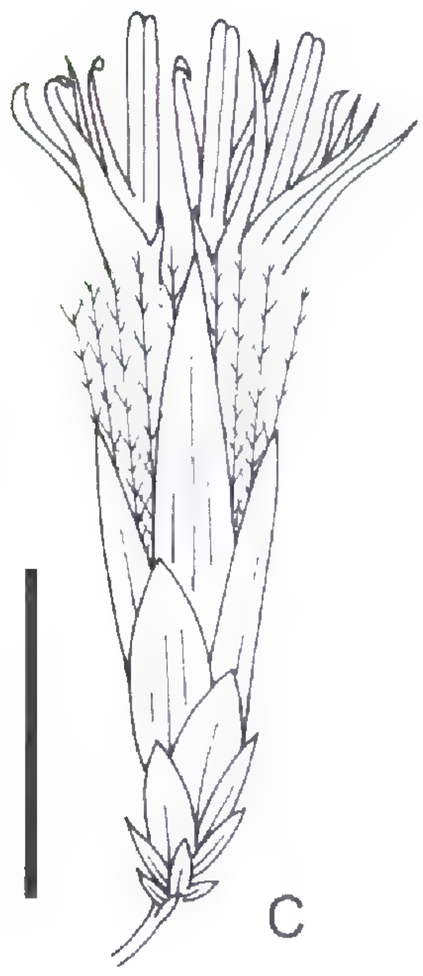

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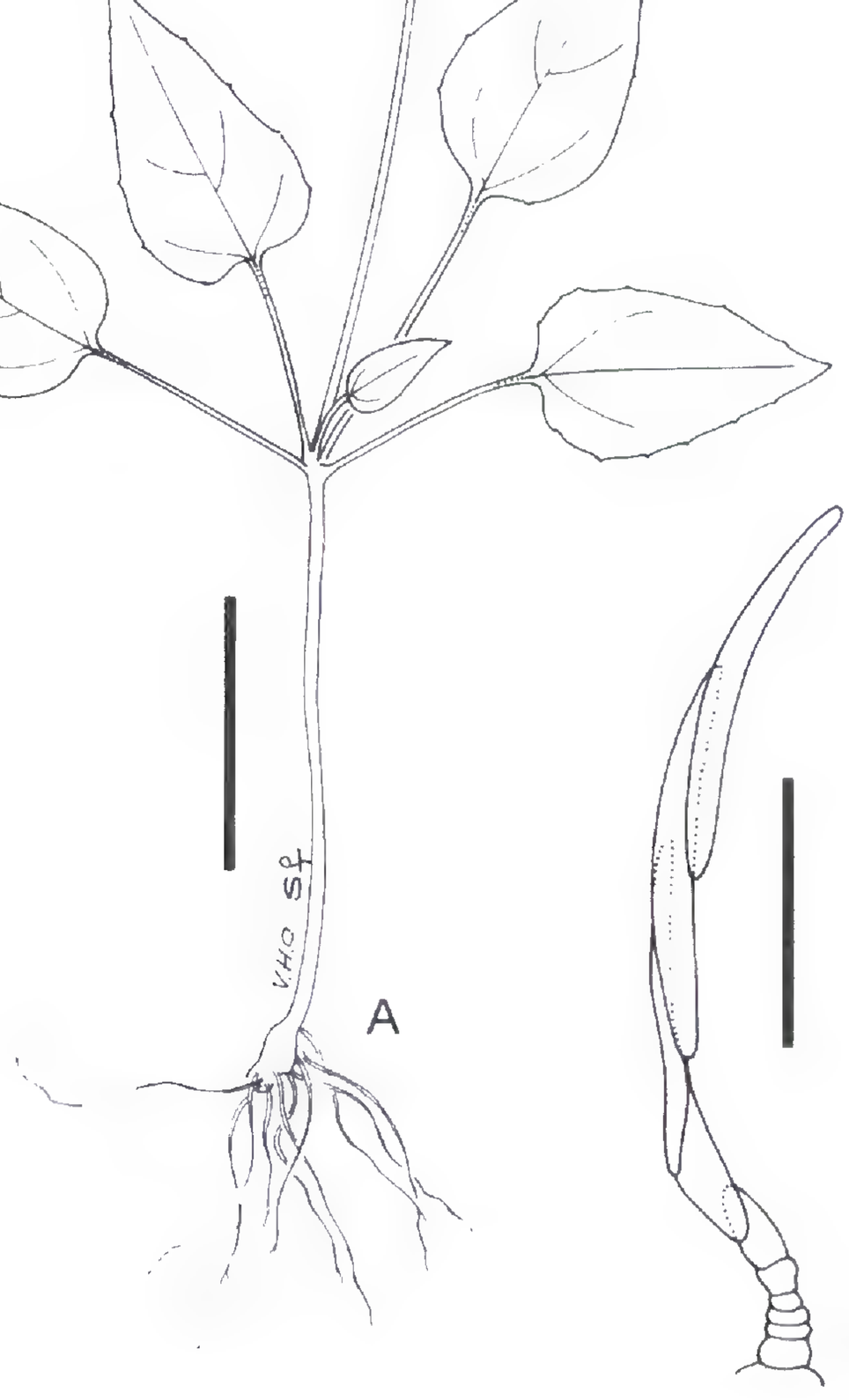


A

B

F

E

Figure 49. *Ainsliaea gracilis* Franch. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Chasmogamous floret. —E. Stamen of chasmogamous floret. —F. Style branches of chasmogamous floret. —G. Cleistogamous floret. Scale bars: A = 3 cm; B = 0.2 mm; C = 5 mm; D, G = 2 mm; E, F = 1 mm. Drawn from A–F, *Sino-Amer. Exped. 1970* (UC); G, *Wilson 1811* (W).

Relationships. *Ainsliaea gracilis* resembles *A. kawakamii*, sharing leaf blades ovate, with the margins obscurely dentate and the achenes glabrous or apically pilose. However, *A. gracilis* may be distinguished by its leaf blades acuminate (vs. obtuse to acute) and purplish green on lower surface (vs. pale green).

Representative specimens examined. CHINA. **Guangdong:** Lian Xian, *P. X. Tan 59820* (MO). **Guangxi:** Guangxi, *S. Dayao Team 12856* (MO); "Kwangsi," Na Yan Ts'o, Hsi-chang village & vicinity, Ch'i-fen-shan, Kwei-lin, *W. T. Tsang 28488* (US); 12 Oct. 1936, *s. coll. 40018* (MO). **Guizhou:** "Kweichow," Yinkiang, *Y. Tsiang 7913* (NY). **Hubei:** "Hupeh," *E. H. Wilson 1811* (NY, W); Western Hubei, *Metasequoia* region of Lichuan Xian (Hsien), vicinity of Lojiaba on the W side of the valley, *B. Bartholomew et al. (Sino-Amer. Exped.) 1970* (CM, UC), vicinity of Zhuanjiaowan on the E side of the valley, *B. Bartholomew et al. (Sino-Amer. Exped.) 2041* (UC). **Hunan:** Ma-Ling-Tung, Sinning Hsien, *C. S. Fan & Y. Y. Li 621* (W). **Locality unknown:** "China," *F. Wei 1453* (MO); 25 Sep. 1942, *s. coll. 83689* (MO 04105624); s.d., *s. coll. 941166* (MO 04199602).

7. Ainsliaea grossedentata Franch., J. Bot. (Morot) 8(17): 297. 1894. TYPE: China. Sichuan: Sutchuen oriental, Tchen-keou-tin, 2000 m, *R. P. Farges 333* (holotype, P not seen; isotype, P!). Figure 50.

Ainsliaea gracilis var. *robusta* Diels, Bot. Jahrb. Syst. 29(5): 629. 1901. Syn. nov. TYPE: China. Sichuan: Nan-ch'uan, Tangai-kuo, 12 Sep. 1891, *C. Bock & A. v. Rosthorn 877* (type, O!).

Plants perennial, herbaceous, 0.35–0.60 m tall; stems erect, unbranched, naked, glabrous. Leaves clustered above the base, falsely verticillate; longly petiolate, petioles wingless, 2–4.5 cm, flat, glabrous; leaf blades chartaceous, ovate, 2.8–4.5(6) × 2 4(4.5) cm, base subcordate to truncate, apex subobtuse, margins shallowly pinnately lobed to dentate; slightly discolorous, upper surface subglabrous, and lower surface paler, loosely strigose with long straight trichomes; palmati-pinnate, 3-nerved. Capitula subsessile, arranged in a stachyoid inflorescence; peduncles bracteolate; involucre 5- to 6-seriate, 7–8 × ca. 2 mm, cylindrical; phyllaries papyraceous, apically pilose; outer phyllaries ovate, obtuse, ca. 1.5 × 1 mm, inner phyllaries ovate to lanceolate, acute, 3-nerved, 7–8 × ca. 0.5 mm. Florets 3, hermaphrodite, chasmogamous; corolla slightly ligulate, 10–10.5 mm, lobes ca. 4 mm (deeper slit ca. 5 mm), tube 5–5.5 mm, anthers 3–5 mm, apical appendages truncate, ca. 0.5 mm, sagittate; tails ca. 1.2 mm, shortly pilose; style branches obtuse to acute, ca. 0.5 mm. Achenes linear to oblong, ca. 2 mm, 10-nerved, glabrous; pappus bristles ca. 40, ca. 6 mm.

Distribution and phenology. China (Guizhou, Hubei, Sichuan); collected from elevations between 1300 and 2000 m (Fig. 4B); flowering in September.

Discussion. *Ainsliaea gracilis* var. *robusta* is proposed as a new synonym of *A. grossedentata* by its leaf margins shallowly pinnately lobed to dentate (vs. obscurely denticulate in *A. gracilis*). In addition, the differential character mentioned in its protologue, i.e., larger plants, is normal in the specimens cited here.

Vernacular name. Pao chie tsao (C. Bock & A. V. Rosthorn 877, O).

Relationships. *Ainsliaea grossedentata* resembles *A. apiculata* from Japan in its leaves and anthers. Both have lobate leaves and rounded anther appendages. However, *A. grossedentata* may be distinguished by its leaf blades shallowly pinnatilobed (vs. palmately lobed) and achenes glabrous (vs. achenes pilose).

Representative specimens examined. CHINA. **Guizhou:** "Kweichow," Niu Tou Shan, Fan Ching Shan, *A. N. Steward et al. 418* (NY, S, US). **Hubei:** "W Hupeh," *E. H. Wilson 2743* (NY, W); "Hupeh," *A. Henry 6637A* (US). **Sichuan:** "Sut chuen oriental, Tchen kéou tin" [Chengkou], s.d., *R. P. Farges s.n.* (US 2492364).

8. Ainsliaea kawakamii Hayata, Icon. Pl. Formos. 8: 72. 1919. TYPE: China. Taiwan: Formosa, *T. Kawakami s.n.* (holotype, TI not seen; isotype, TI photo LP!). Figure 51.

Ainsliaea hui Diels ex Mattf., Notizbl. Bot. Gart. Berlin-Dahlem 11: 109. 1931. Syn. nov. TYPE: China. Zhejiang: Tschekiang, Hangchow, Drageon, 14 Oct. 1927, *R. C. Ching 3861* (type, W!).

Plants perennial, herbaceous, 0.20–0.70 m tall; stems many or single, erect, unbranched (occasionally few branched at the base of the inflorescence), naked. Leaves clustered, 4–5 cm above stem base or crowded near the midpoint of stem, long-petiolate; petioles wingless, (2.5)3.5–8.5 mm; leaf blades ovate, chartaceous, 5–7(9.5) × 3–6 cm, apex obtuse, base rounded or slightly cordate to truncate, margins slightly entire to denticulate; upper surface subglabrous, lower surface paler, with few long straight trichomes; palmati-pinnate, 3-nerved. Capitula short-peduncu-late, arranged in a stachyoid inflorescence; involucre 6- to 7-seriate, campanulate-cylindrical, 10–13 × ca. 3 mm; phyllaries subcoriaceous, glabrous, sparsely pilose above; outer phyllaries ovate, obtuse, ca. 2.5 × 1.2 mm; inner phyllaries linear, acute, 3-nerved, ca. 7.5 × 1 mm. Florets 3, hermaphrodite, usually chasmogamous (occasionally cleistogamous in the single plant), corollas white; *chasmogamous florets* with corollas ligulate, ca. 13 mm, lobes ca. 6.5 mm (deeper slit ca. 8 mm), tube ca. 5 mm; anthers purple,

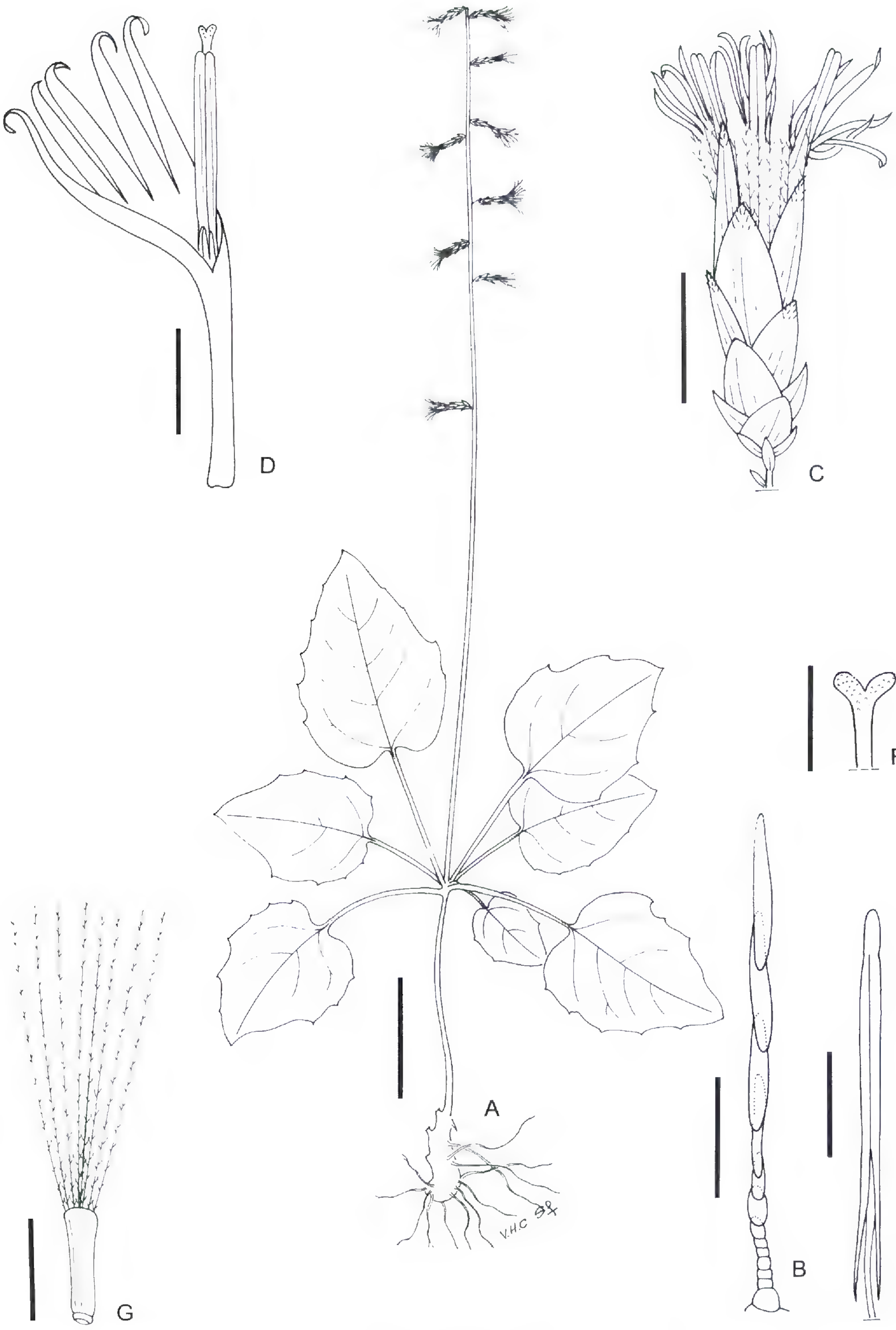


Figure 50. *Ainsliaea grossedentata* Franch. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Chasmogamous floret without pappus and ovary. —E. Stamen. —F. Style branches. —G. Achene with pappus. Scale bars: A = 3 cm; B = 0.2 mm; C = 4 mm; D, G = 2 mm; E, F = 1 mm. Drawn from *Farges s.n.* (US).

Figure 51. *Ainsliaea kawakamii* Hayata. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D, F. Cleistogamous floret: D, corolla; F, achene with pappus. E, G–I. Chasmogamous floret: E, floret without pappus and ovary; G, achene with pappus; H, style branches; I, stamen. Scale bars: A = 3 cm; B = 0.1 mm; C = 5 mm; D = 2 mm; E–G = 5 mm; H = 1 mm; I = 1.5 mm. Drawn from A, the photo of type *Kawakami s.n.* (TI); B, *Ching 2459* (UC); C, E, G–I, *Fang 39* (MO); D, F, *Li 0317* (MO).

ca. 6 mm, apical appendages truncate, ca. 1 mm, sagittate; tails ca. 1.5 mm, shortly pilose; style branches short, rounded, ca. 0.3 mm. Achenes oblong, ca. 2.5 mm, glabrous or pilose above, 10-nerved; pappus bristles 40 to 43, ca. 8 mm; *cleistogamous florets* with corollas tubulose to pseudoligulate, ca. 4.5 mm, 5-lobed, lobes ca. 0.4 mm (deeper slit ca. 1 mm); anthers much reduced, ca. 2 mm; style branches ca. 0.4 mm. Achenes ca. 6 mm; pappus bristles ca. 9 mm.

Distribution and phenology. China (Anhui, Fujian, Guangdong, Hunan, Zhejiang) and Taiwan. It was collected in woods, on slopes between 600 and 1550 m (Fig. 6A); flowering from September to November (flower buds in June and August).

Observations of the type material. The type material cited in the original diagnosis of *Ainsliaea hui* Diels ex Mattf. corresponds (in locality and date) to that deposited at W, except in the collector number, i.e., "3896" (original diagnosis) instead of "3861" (specimen at W). It is probable that this was a printing error.

Discussion. The original diagnosis of *Ainsliaea kawakamii* mentions achenes as pilose. In contrast, all specimens assigned there (including materials with immature capitula, e.g., *Ching 2459*, *Steward 7163*) have achenes glabrous or apically pilose only. I was not able to see the type material at TI (only its photo) in order to confirm the original diagnosis. However, other characters, such as the leaf blades ovate or ovate to deltoid, rounded to truncate at the base and obtuse at the apex, with leaf margins entire to slightly denticulate, are considered diagnostic for the species. Although *A. kawakamii* was said to have pilose achenes and *A. hui* has apically pilose achenes, since no other diagnostic character that can separate them as distinct taxa was found, *A. hui* is proposed as a new synonym of *A. kawakamii*.

Relationships. *Ainsliaea kawakamii* resembles *A. gracilis*, with ovate leaf blades, margins either entire or slightly denticulate, and the achenes glabrous or apically pilose. *Ainsliaea kawakamii* may be distinguished by its leaf blades acute to obtuse at the apex (vs. acuminate) and paler green below (vs. purplish green).

Representative specimens examined. CHINA. **Anhui:** "Anhwei," Huang Shan, *R. C. Ching 4133, herb. 8524* (UC); "Kuan Sze-Wang Shan," *A. N. Steward 1292, herb. 7163* (UC, US). **Fujian:** Nanping, *G. He 4125* (MO). **Guangdong:** Canton, *L. Deng 6306* (MO); Canton, *L. Deng 1449* (MO), *G. Ding & G. Shi 1087* (MO); Canton, *X. P. Gao 53659* (MO); Canton, *Z. Li 317* (MO); Ruyuan, *C. Wang 12244* (MO). **Hunan:** *S. Q. Chen 2693* (MO). **Zhejiang:** southern Chekiang, region of King Yuan, *R. C. Ching 2459* (NY, UC, US); Hangzhou, *Fang 38* (MO), *Fang 39* (MO); Zhejiang, *S. Y. Ho 7190* (MO); Hangzhou, *Y. Jin 382* (MO); Chekiang, Lien-Tsi-Shan, *Y. L. Keng 1019* (paratype of *Ainsliaea hui*, UC); Tien-mu-shan, *K. Yoa et al. 79344* (CM). TAIWAN. Hsinchu Co., Wutzushan, *K. C. Yang 791* (CM). **Locality unknown**. *Z. Liu 28894* (MO); *X. Liu 25245* (MO); *Z. Wei 120645* (MO); *s. coll. 40304* (PE 26505).

9. Ainsliaea linearis Makino, Bot. Mag. 23(275): 250. 1909 (20 Dec. 1909). TYPE: Japan. Kyushu. Kagoshima: Osumi, Yakushima, Onaida, 10 Dec. 1956, *M. Togasi 1448* (neotype, designated here, MO; duplicates, CM!, NY!, S!, TNS not seen, UC!, US!, W!). Figure 52.

Ainsliaea faurieana Beauverd, Bull. Soc. Bot. Genève, Sér. 2: 381. 1909. TYPE: Japan. Kyushu. Kagoshima: Yakushima, Liukiu, "in petrosis torrentium," July 1900, *U. Faurie 4065* (type, KYO!).

Plants perennial, herbaceous, 0.10–0.3(0.50) m tall; stems 1 to many, erect, unbranched (occasionally branched above the leaves), loosely strigose. Leaves clustered at the median part of the stem, long-petiolate, petioles narrowly winged, subglabrous, 1–3 cm; leaf blades chartaceous, linear to elliptic, 2.5–5.5 × 0.3–0.8 cm, gradually attenuated at the base, acute mucronate at the apex, margins slightly crenate, revolute, with 4 to 8 teeth on each side; obscurely palmati-pinnate, apparently 1-nerved (midvein prominent); slightly discolorous, subglabrous on both surfaces, with long straight trichomes. Capitula short-pedunculate, arranged in thyrsoid inflorescence; peduncles 1–4 mm, bracteolate, the upper bracteoles transitioning to involucral bracts or phyllaries; involucre 5- or 6-seriate, cylindrical, 7–10 × 1.5–2 mm; phyllaries papyraceous, margins membranous, obtuse and mucronate at the apex, usually purpurescent toward the tip, glabrous, 3- to 5-nerved; outer phyllaries ovate, ca. 2 × 0.8 mm; inner phyllaries linear to elliptic or linear, 6–7 × 0.5–1.2 mm wide. Florets 3(5), hermaphrodite, chasmogamous; corolla white, ligulate, 6–8.5 mm, 5-lobed, lobes ca. 1.5 mm (deeper slit ca. 2.5 mm), tube 3–5 mm; anthers ca. 2.5 mm, apical appendages emarginate, ca. 0.5 mm, tails ca. 1 mm, shortly pilose; style branches rounded, ca. 0.5 mm long. Achenes linear-oblong, 2–4 mm, 10-nerved, densely pubescent; pappus bristles ca. 40, 5–6(10) mm.

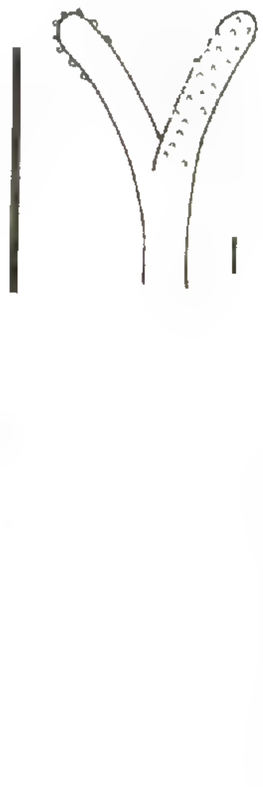
Distribution and phenology. Japan (Yakushima); collected from mossy sites in forests, along streams, and among rocks of stream beds below high water line, at elevations between 350 and 1400 m (Fig. 5B); flowering from August to October and in December.

Discussion. The type designated in the protologue of *Ainsliaea linearis*, "Prov. Osumi: Yaku-shima (T. Makino! Sept. 1909)," has not been found at KYO, MAK, or TI, where the original herbarium of Makino should be, and no illustration was published with the name. The collection *M. Togasi 1448* kept at MO, which is a widely distributed collection (duplicates seen at CM, NY, S, TNS, UC, US, W) and agrees in all respects with the protologue of *A. linearis*, is here chosen as the neotype.

Vernacular name. Hosoba-haguma (Makino, 1909; Masamune, 1934; Kitamura, 1965).

Relationships. *Ainsliaea linearis* is closely related to *A. walkeri* from China in its habit and capitula. Both species have verticillate leaves, leaf blades that are linear to elliptic, with narrowly alate petioles and capitula arranged in spiciform panicles. *Ainsliaea*

F



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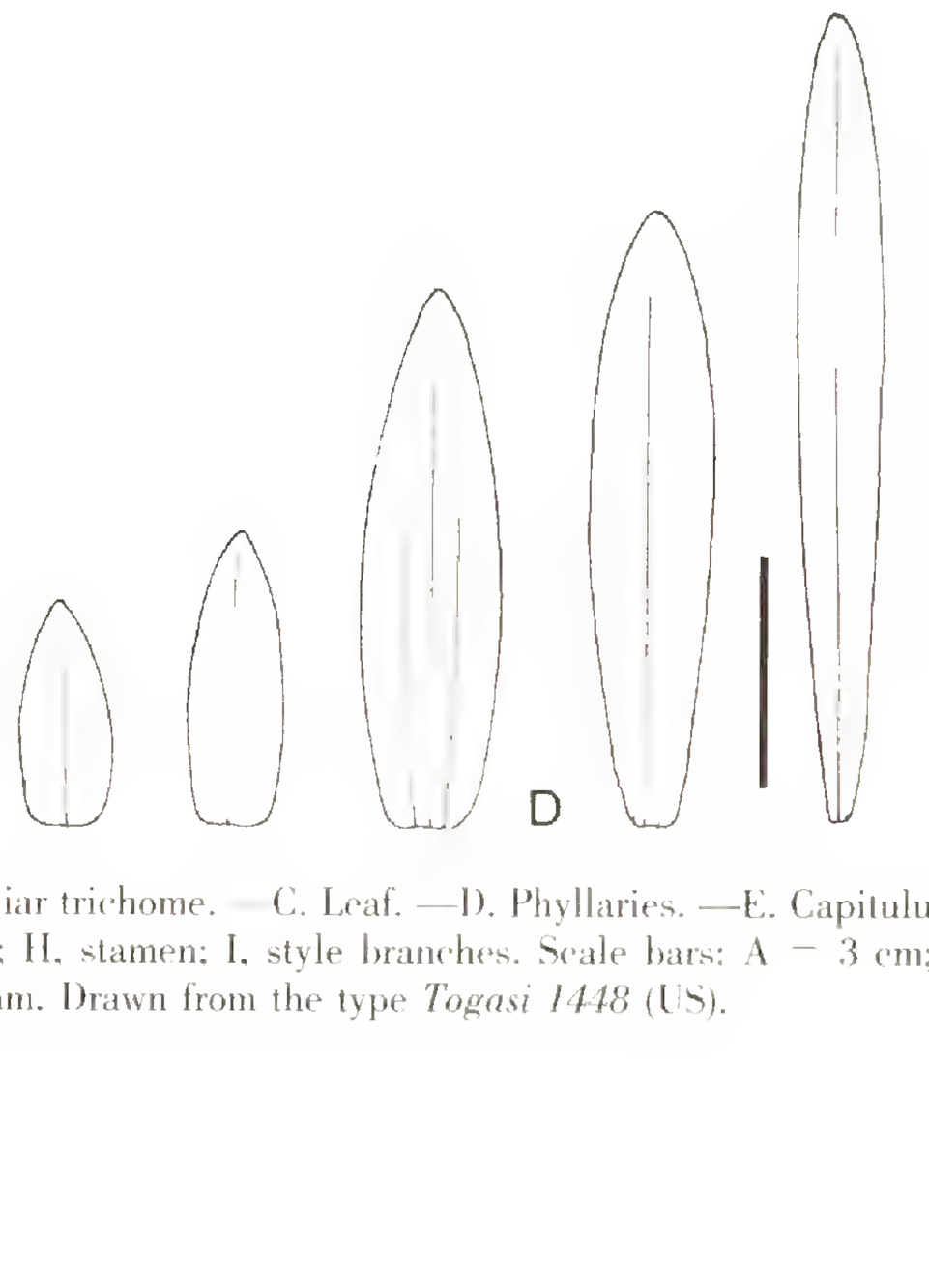

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Figure 52. *Ainsliaea linearis* Makino. —A. Habit. —B. Foliar trichome. —C. Leaf. —D. Phyllaries. —E. Capitulum. —F–I. Chasmogamous floret: F, achene with pappus; G, corolla; H, stamen; I, style branches. Scale bars: A = 3 cm; B = 0.1 mm; C = 1 cm; D, F, G = 2 mm; E = 5 mm; H, I = 1 mm. Drawn from the type *Togasi 1448* (US).

linearis may be distinguished by its leaf margins that are crenate with four to eight teeth on each side (vs. margins with only one or two teeth on each side toward the apex).

Representative specimens examined. JAPAN. Kyushu. **Kagoshima:** Isl. Yakushima, Yaku-cho, downstream from Yodoko Hut along the Yodoko River, *D. E. Boufford & S. Mitsuta 20062* (CM); Isl. Yakushima, en route from Yodogogoya Hut to Onoaida along Onoaida Path, *K. Iwatsuki et al. 113* (CANB); Yakushima, *G. Masamune in 1927* (NY); Isl. Yakushima, along point R-A-B-C, in Hanayama reserved forest, near My. Yakéminé, Yaku-choh, along trail, *S. Mitsuta & H. Doei 411* (TI); Isl. Yakushima, Osumi Gunto, Tachudake, Jigyosho, *R. Moran 5322* (UC); Isl. Yakushima, along Hirase Forestry Road, N slope of Mt. Yoshida-dake, *G. Murata et al. 15480* (AAU); Isl. Yakushima, around Sempiro Waterfall, along Tainokawa River, *G. Murata et al. 15716* (TI); Isl. Yakushima, prope Miyanoura, *Tagawa & Motozi 1970* (NY).

10. Ainsliaea macroclinidioides Hayata, J. Coll. Sci. Imp. Univ. Tokyo 25, 19: 141. 1908. TYPE: Taiwan. Suizan, 7702 ft., Oct. 1905, *Nagasawa 665* (lectotype, designated here, TI not seen; duplicate, TI photo LP!). Figure 53.

Ainsliaea okinawensis Hayata, J. Coll. Sci. Imp. Univ. Tokyo 30: 161. 1911. *Ainsliaea macroclinidioides* Hayata var. *okinawensis* (Hayata) Kitam., J. Jap. Bot. 20(4): 192. 1944. TYPE: Japan. Pref. Okinawa, Apr. 1887, *Z. Tashiro s.n.* (holotype, TI not seen; isotype, TI photo LP!).

Ainsliaea ovata Koidz., Bot. Mag. 28: 150. 1914. TYPE: Japan. Pref. Okinawa: Isl. Okinawa, Liukiu, Sep. 1889, *Tashiro s.n.* (holotype, TI not seen; isotype, TI photo LP!).

Ainsliaea dentata Koidz., Bot. Mag. 28: 149. 1914. TYPE: Japan. Pref. Okinawa: Isl. Okinawa, Liukiu (lectotype, designated here, TI not seen; duplicate, TI photo LP!).

Ainsliaea yadsimae Koidz., Bot. Mag. 28: 149. 1914. TYPE: Japan. Pref. Okinawa: Isl. Okinawa, Liukiu (lectotype, designated here, TI not seen; duplicate, TI photo LP!).

Plants perennial, herbaceous, 0.25–0.80 m tall; stems erect, unbranched, single (occasionally 3 or 4), usually naked, loosely strigose. Leaves clustered at the median part of the stem, long-petiolate; petioles wingless, flat, 3–11 cm; leaf blades chartaceous, ovate to lanceolate or ovate to deltoid, 4.5–13 × 3–11 cm, apex acute or acuminate to longly acuminate, base rounded or slightly to deeply cordate, margins from obscurely dentate to dentate; slightly discolorous, upper surface subglabrous, lower surface loosely strigose, with long straight trichomes; palmati-pinnate, 3- to 5-nerved. Capitula short-pedunculate, arranged in a stachyoid inflorescence; peduncles bracteolate, 2–4 mm; involucre 3- or 4(6)-seriate, cylindrical, 9–11 × 3–5 mm, phyllaries papyraceous, glabrous; outer phyllaries broadly ovate, acute, 1.5–2.5 × ca. 1 mm; inner phyllaries oblanceolate, acute, conspicuously 3- to 5-nerved, 9–10 × ca. 1.5 mm. Florets 2 or 3, hermaphrodite, chasmogamous; corollas white, ligulate, 9–9.5 mm, lobes 3–3.5 mm (deeper slit ca. 5 mm), tube 4–4.5 mm; anthers ca. 5 mm, apical appendages rounded, ca. 0.5 mm, sagittate; tails ca. 1.5 mm, shortly pilose; style branches short, rounded, ca. 0.4 mm. Achenes oblong-obovate, 3–6 mm, 10-nerved, pilose; pappus bristles 35 to 50, 6–9 mm.

Distribution and phenology. Japan (Ryukyu archipelago) and Taiwan. It was collected in evergreen forests along rivers, from near sea level to ca. 2000 m (Fig 6A); flowering in September, October, and from December to April (flower bud in July and August).

Discussion. Two syntypes are mentioned in the protologue of *Ainsliaea macroclinidioides*, "Suizan, ad 7702 ped. alt., *leg. Nagasawa*, Oct. 1905, *(No. 665)*" (TI photo at LP!) and "Kagi: Tappansha, *leg. T. Kawakami et U. Mori*, Oct. 1906, *(No. 1971)*" (photo at A!). I propose the material "*Nagasawa 665*," which presents the most complete plant, as lectotype of *A. macroclinidioides*.

The authors follow the opinion of H. Ohba (pers. comm., 23 Apr. 2002) in the selection of the lectotypes for *Ainsliaea dentata* and *A. yadsimae*.

Numerous specific taxa have been proposed to account for the variability that occurs around *Ainsliaea macroclinidioides* (see synonymy). *Ainsliaea ovata* was described on the basis of its ovate leaves that were deeply to slightly cordate at the base, with denticulate leaf margins. *Ainsliaea dentata* was based on its ovate leaves that were rounded at the base, with dentate leaf margins. *Ainsliaea yadsimae* was said to be differentiated by its ovate leaves that were rounded at the base, with slightly dentate leaf margins, and *A. okinawensis* on the basis of its ovate leaves, acuminate at the apex and cordate to rounded at the base, with slightly denticulate leaf margins. However, subsequent collections have shown these leaf characters to be within the range of variation for the species.

Ainsliaea macroclinidioides is treated here as a polymorphic species, with leaf blades varying from lanceolate to deltoid, long-acuminate to acuminate at the apex, with rounded to deeply cordate bases, and margins dentate to obscurely dentate.

Vernacular names. Okinawa-haguma (Koidzumi, 1914), Oh-Okinawahaguma (Koidzumi, 1914), Maruba-haguma (Koidzumi, 1914), Nakahara-haguma (Kitamura, 1944), Okinawa teishoso (*Tetsuo Amano 6903*, KYO).

Representative specimens examined. JAPAN. Ryukyu. **Okinawa: Isl. Amami-Ohshima.** *S. Kawagoe in 1919* (US); Loochoo curve, *H. Obha in 1924* (KYO). **Isl. Iheyajima**, Mt. Gayo, *T. Amano 7485* (KYO). **Isl. Iriomote**, Mt. Goza, *K. Iwatsuki et al. 517* (MO); Yaeyama-gun,

Figure 53. *Ainsliaea macroclinidioides* Hayata. —A. Habit. —B–D. Leaves from different specimens showing the variation in the leaf blades. —E. Foliar trichome. —F. Capitulum. G. Chasmogamous floret without pappus and ovary. —H. Style branches. —I. Stamen. —J. Achene with pappus. Scale bars: A–D = 3 cm; E = 0.2 mm; F = 5 mm; G, J = 2 mm; H, I = 1 mm. Drawn from B, photo of type *A. macroclinidioides* Hayata, *Nagasawa 665* (TI); A, E–J, *Iwatsuki et al. 15* (KYO); C, *Kanashiro 23* (KYO); D, *Murata 17066* (AAU).

Taketomi-cho, the upper part of Kakara river, *M. Kato et al. 151* (KYO); upper stream of Urauchigawa, *N. Kurosaki 5676* (KYO); Yaeyama-gun, the upper area of Urauchi river Taketomi-cho, *H. Okada et al. 235* (KYO); near Urauchigawa, *H. Koyama et al. 237* (KYO); S side of Mt. Goza-dake, *M. Tagawa & K. Iwatsuki 4655* (KYO); Iriomote-jima, Ootomi Riv. Urauchigawa, *Y. Tateishi & G. Murata 4770* (TI). **Isl. Irumti**, *G. Koidzumi in 1923* (KYO). **Isl. Ishigaki**, Kabira, *T. Amano 6903* (KYO); Mt. Omoto, *K. Iwatsuki et al. 556* (KYO); Mt. Omoto-dake, *G. Murata 17066* (AAU). **Isl. Kumejima**, *S. Tawada 39* (KYO). **Isl. Okinawa**, Kunigami, Onna-dake, *T. Amano 6874* (KYO, UC, US); near summit of Ishakawa Take, *J. T. Conover 2830* (US); Kunigami-gun, Mt. Yonaha, *K. Iwatsuki et al. 15* (KYO, TI); Kunigami, Pueblo Hanechi, Mt. Tanyu, *T. Kanashiro 23* (KYO); Ohgimi-son, *T. Kanashiro 2004* (KYO, US); Mt. Genka, *K. Miyagi in 1911* (KYO); Kunigami, s.d., *K. Miyasato s.n.* (KYO); Mt. Taniudake, *T. Naito in 1927* (US); Kunigami, *S. Sakaguchi in 1922* (KYO); Mt. Hennoki, *S. Sonohara 642* (KYO); Kunigami, Hendona, s.d., *S. Sonohara s.n.* (KYO); Kunigami, Tanyu-dake, *S. Sonohara et al. 6286* (KYO, US); Insl. Utchina, Kunigami, Pueblo Satefukin, *S. Sonohara in 1922* (KYO); Mt. Okuno, *Z. Tashiro in 1924* (KYO); Mt. Sate, *Z. Tashiro in 1923* (KYO); Kunigami, Pueblo Kunigami, *S. Tawada 29* (KYO); Motobu Peninsula, foot of Mt. Awa, *E. H. Walker 7631* (US). **Isl. Tanegashima**, Nishino omote, *s. coll. 11* (KYO). **Isls. Tokara,** Isl. Akuseki, *S. Sako 6216* (KYO). TAIWAN. Taipei Hsien Co., forest to N. Cha Tien Mt., San Hsie, *T. H. Hsieh & C. H. Hsiao in 1988* (NY); Chiayi Co., Funchihu, *S. F. Huang 2588* (MO); Mt. Niitaka, *R. Kanehira & S. Sasaki 21765* (UC, US); Kagi, Tappanasha, *T. Kawakamii & U. Mori 1971* (paratype of *A. macroclinidioides*, TI not seen; TI photo A!).

11. Ainsliaea oblonga Koidz., Bot. Mag. (Tokyo) 28(329): 150. 1914. *Ainsliaea macroclinidioides* var. *oblonga* (Koidz.) Hatus., Flora of the Ryukyu: 607. 1975. TYPE: Japan. Yaeyama archipelago, insl. Nisiomotesima, Aug. 1889. *Tashiro s.n.* (holotype, TI not seen; isotype, TI photo LP!). Figure 54.

Plants perennial, herbaceous, 0.25–0.35 m tall; stems erect, simple or branched above the leaves, subglabrous. Leaves clustered at the midpoint of the stem, long-petiolate; petioles wingless, flat, glabrous, 2–6 cm; leaf blades chartaceous, lanceolate, base attenuate to cuneate; slightly discolorous, upper surface deep green, subglabrous, and lower surface paler, loosely strigose with long straight trichomes; palmati-pinnate, 3-nerved. Capitula shortly pedunculate, arranged in thyrsoid inflorescence; peduncles bracteolate, with upper bracts transitioning to involucre, 2–4 mm; involucre 6- or 7-seriate, ca. 10 × 3.5 mm, cylindrical; phyllaries papyraceous, glabrous; outer phyllaries ovate, acute, ca. 1.2 × 0.8 mm; inner phyllaries linear to lanceolate, acute, 3-nerved, ca. 9 × 0.8 mm. Florets 3, hermaphrodite, chasmogamous; corolla ligulate, 9–9.5 mm, lobes 3.5–4 mm (deeper slit ca. 5 mm), tube 4–4.5 mm; anthers ca. 4 mm, apical appendages rounded to truncate, ca. 0.7 mm, sagittate; tails ca. 1 mm, shortly pilose; style branches rounded to truncate, ca. 0.7 mm. Achenes oblong to elliptic, ca. 5.5 mm, 10-nerved, densely pilose; pappus bristles ca. 36, ca. 7 mm.

Distribution. China (Guangdong, Guangxi) and Japan (Ryukyu Islands) (Fig. 6B).

Key to the varieties of Ainsliaea oblonga *in China and Japan*

1a. Leaf margins obscurely denticulate; leaf blades lanceolate, acute to acuminate at the apex . . . . . 11a. var. *oblonga*

1b. Leaf margins dentate; leaf blades broadly lanceolate, acute at the apex . . . . . 11b. var. *latifolia*

11a. Ainsliaea oblonga Koidz. var. **oblonga**. Figure 54A–G.

Leaf blades 3–8 × 1–2(2.5) cm, acute to acuminate at the apex, leaf margins obscurely denticulate.

Distribution and phenology. China (Guangdong, Guangxi) and Japan (Yaeyama archipelago); collected in flower in October and January, in flower bud in August and September.

Vernacular names. Hosoba-haguma (Koidzumi, 1914), Nagaba-haguma (*Elliott 1104*, US).

Relationships. Ainsliaea oblonga var. *oblonga* resembles *A. trinervis* from China in its habit and capitula. Both taxa have verticillate leaves, leaf blades that are trinerved, and pedunculate capitula. *Ainsliaea oblonga* var. *oblonga* may be distinguished by its lanceolate leaf blades, ca. 1–2(2.5) cm wide (vs. narrowly lanceolate, ca. 0.5–1.3 cm wide).

Representative specimens examined. CHINA. **Guangdong:** *s. coll. 6207* (MO); Canton, Mt. Kabi, *s. coll. 89755* (MO). **Guangxi:** "Kiangsi," Sai Hang Cheung, Kiennan District, *S. K. Lau 4041* (US). **Locality unknown:** *s. coll. 55769* (MO). JAPAN. Ryukyu. **Okinawa:** Taira-ku, Kushi Son; Kunigami-Gun, Okinawa-Shima, *J. C. Elliott 1104* (US).

11b. Ainsliaea oblonga Koidz. var. **latifolia** Kitam., J. Jap. Bot. 14: 302. 1938. *Ainsliaea macroclinidioides* var. *latifolia* (Kitam.) Hatus., Flora of the Ryukyu: 607. 1975. TYPE: Japan. Kagoshima Prefecture: Insl. Amami-oshima, Mt. Yuwan, Yuwandake, Nov. 1925, *H. Ohba* (holotype, KYO not seen; isotype, KYO photo LP!). Figure 54H.

Leaf blades 8–11 × 3.5–4.5 cm, subobtuse to acute at the apex. Leaf margins dentate.

Distribution and phenology. At present it is only known from the type collection from Amami-oshima Island; flowering in November.

Figure 51. *Ainsliaea oblonga* Koidz. A–G. *A. oblonga* var. *oblonga*. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D–G. Chasmogamous floret: D, corolla; E, stamen; F, style branches; G, achene with pappus. —H. *A. oblonga* var. *latifolia* Kitam. Leaves. Scale bars: A, H = 3 cm; B = 0.1 mm; C = 5 mm; D, G = 2 mm; E, F = 1 mm. Drawn from *Elliot 1140* (US).

Relationships. The var. *latifolia* resembles *Ainsliaea macroclinidioides* in its leaves with dentate margins. *Ainsliaea oblonga* var. *latifolia* may be distinguished by its leaf blades that are broadly elliptic (vs. ovate to deltoid or ovate to lanceolate) and attenuate at the base (vs. rounded to slightly cordate).

12. Ainsliaea parvifolia Merr., Philipp. J. Sci. 12: 110. 1917. TYPE: China. Guangdong: Loh Fau Mountain (Lofaushan), 28 Oct. 1916, *E. D. Merrill 10237* (holotype, US!). Figure 55.

Plants perennial, herbaceous, 0.11–0.25 m tall: stems erect, unbranched, naked, sparsely strigose. Leaves basally clustered, rosulate, long-petiolate: petioles wingless, 2–5 cm, flat, strigose, leaf blades subcoriaceous, ovate or oblong to ovate, 2–3.5 × 1.5–2 cm, apex acute-mucronate, base abruptly rounded, then cuneately narrowed into the petiole, margins denticulate; adaxial surface subglabrous, abaxial surface moderately strigose, with long straight trichomes; palmati-pinnate, 3-nerved. Capitula short-pedunculate, arranged in a stachyoid inflorescence; peduncles with minute bracteoles, 1–3 mm; involucre 5- or 6-seriate, narrowly campanulate, 8–10 × ca. 4 mm; phyllaries subcoriaceous, dull purple, glabrous; outer phyllaries ovate, obtuse, 1–2 × ca. 1 mm; inner phyllaries linear to elliptic, acute, 3-nerved, ca. 10 × 1 mm. Florets 3, hermaphrodite, chasmogamous; corollas white, ligulate, lobes 4–6 m (deeper slit 0.5–1 mm), tube 4.5–5 mm; anthers ca. 5 mm, apical appendages truncate, ca. 0.7 mm, sagittate; tails ca. 1.5 mm, smooth to slightly pilose; style branches shortly bilobate, rounded, ca. 0.3 mm. Achenes oblong to obovate, ca. 2 mm, 10-nerved, apically pilose; pappus bristles ca. 40, 6.5–8 mm.

Distribution and phenology. At present it is known only from the type collection from Lofaushan; found on open grassy slopes between 500 and 1000 m; flowering in October.

Relationships. *Ainsliaea parvifolia* exhibits some likeness to *A. yunnanensis* in its habit and capitula. Both species have leaves long-petiolate, with leaf blades that are ovate and cuneate at the base, and the capitula sessile. *Ainslaea parvifolia* is distinguished by its foliar trichomes, i.e., straight trichomes (vs. T-shaped trichomes), and its achenes apically pilose (vs. pilose).

13. Ainsliaea trinervis Y. C. Tseng, Acta Phytotax. Sin. 31(4): 367. 1993. TYPE. China. Guangxi: Rongshui, *S. Q. Chen 16409* (holotype, IBSC not seen; isotypes, TI not seen, TI photo LP!). Figure 56.

Plants perennial, herbaceous, 0.30–0.60 m tall; stems erect, unbranched, loosely strigose. Leaves clustered at the median part of the stem, petiolate, petioles wingless, flat, pilose, 1–1.5 cm; leaf blades chartaceous, lanceolate, 5–9 × 0.5–1.3 cm, base attenuate, slightly decurrent into the petiole, longly acuminate at the apex, margins denticulate; subglabrous on both surfaces with long straight trichomes; palmati-pinnate, 3-nerved. Capitula pedunculate, arranged in thyrsoid inflorescence; peduncles 3–5 mm, pilose; involucre 6- or 7-seriate, cylindrical, 10–12 × 2–4 mm: phyllaries papyraceous; outer phyllaries ovate, acute, 1–3 × 0.8–1.3 mm, apex pilose; inner phyllaries ovate to lanceolate or narrowly elliptic, acute, 3- to 5-nerved, 10–12 × 1.5–2 mm, glabrous. Florets 3, hermaphrodite, chasmogamous; corolla purple, ligulate, ca. 9.5 mm, lobes ca. 4 mm (deeper slit 4.5 mm), tube ca. 5 mm; anthers ca. 5 mm, apical appendages slightly emarginate, ca. 0.5 mm; tails ca. 1.5 mm, pilose; style branches short, truncate, ca. 0.5 mm. Achenes oblong, ca. 3 mm, 10-nerved, densely pilose; pappus bristles 36 to 38, ca. 7 mm.

Distribution and phenology. China (Guangdong, Guangxi, Guizhou), collected on stony mountain places, in dense trees; fruiting in November, flowering collection without month.

Relationships. *Ainsliaea trinervis* resembles *A. walkeri* in its habit and capitula. Both species have verticillate leaves clustered near the midpoint of stems, leaf blades that are elliptic, and the capitula paniculate. *Ainsliaea trinervis* may be distinguished by its leaf blades 3-nerved (vs. 1-nerved), longly acuminate at the apex (vs. obtuse to acute), margins uniformly denticulate (vs. with only one or two teeth on each side toward the apex), and wingless petioles (vs. narrowly alate).

Representative specimens examined. CHINA. **Guangdong:** Renhua, *L. Deng 7582* (paratype, MO); Ruyuan County, *X. Liu 28990* (MO). **Guangxi:** Damiao Mt., Antai district, Xiaosang village (hill Yuanbao), *S. Q. Chen 17037* (MO); Rongshui, *S. Q. Chen 16610* (paratype, MO), *14449* (paratype, IBSC not seen); Longsheng, *S. L. Yu 700527* (paratype, IBSC not seen), *Exped. Longsheng 50095, 50175* (paratypes, IBSC not seen). **Guizhou:** Rongjiang, *Exped. Qiannan 3261* (paratype, IBSC not seen).

14. Ainsliaea walkeri Hook. f., Bot. Mag. 102: tab. 6225. 1876. TYPE. China. Guangdong: Hong Kong, Dec. 1875, *Capt. A. L. Wilkes s.n.* (holotype, K!). Figure 57.

Plants perennial, herbaceous, 0.15–0.35 m tall; stems erect, unbranched below the inflorescence, scarcely strigose. Leaves clustered at the median part of the stem, petiolate, petioles narrowly winged,

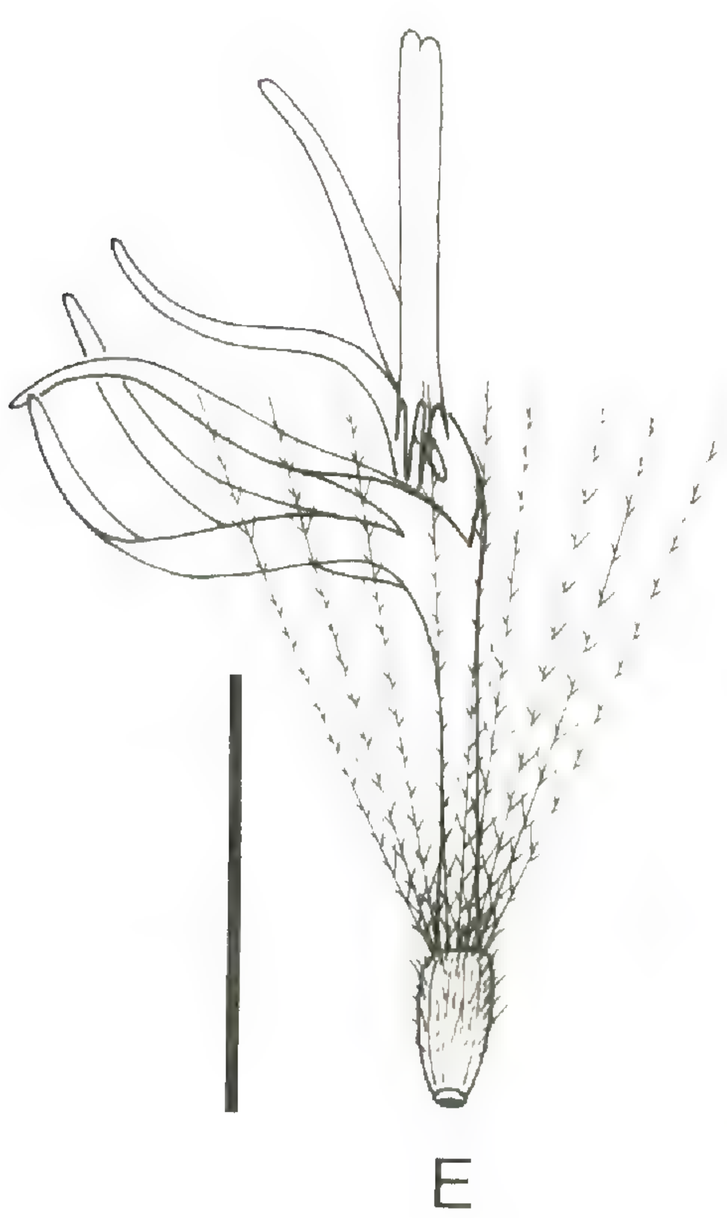

Figure 55. *Ainsliaea parvifolia* Merr. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Phyllaries. —E. Chasmogamous floret. —F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 0.1 mm; C–E = 5 mm; F = 1.5 mm; G = 1 mm. Drawn from the holotype *Merrill 10237* (US).

Figure 56. *Ainsliaea trinervis* Y. C. Tseng. —A. Habit. —B. Foliar trichome. —C. Capitulum. —D. Phyllaries. —E. Chasmogamous floret. —F. Stamen. —G. Style branches. Scale bars: A = 3 cm; B = 0.2 mm; C = 5 mm; D, E = 4 mm; F, G = 1 mm. Drawn from the paratype *Chen 16610* (MO).

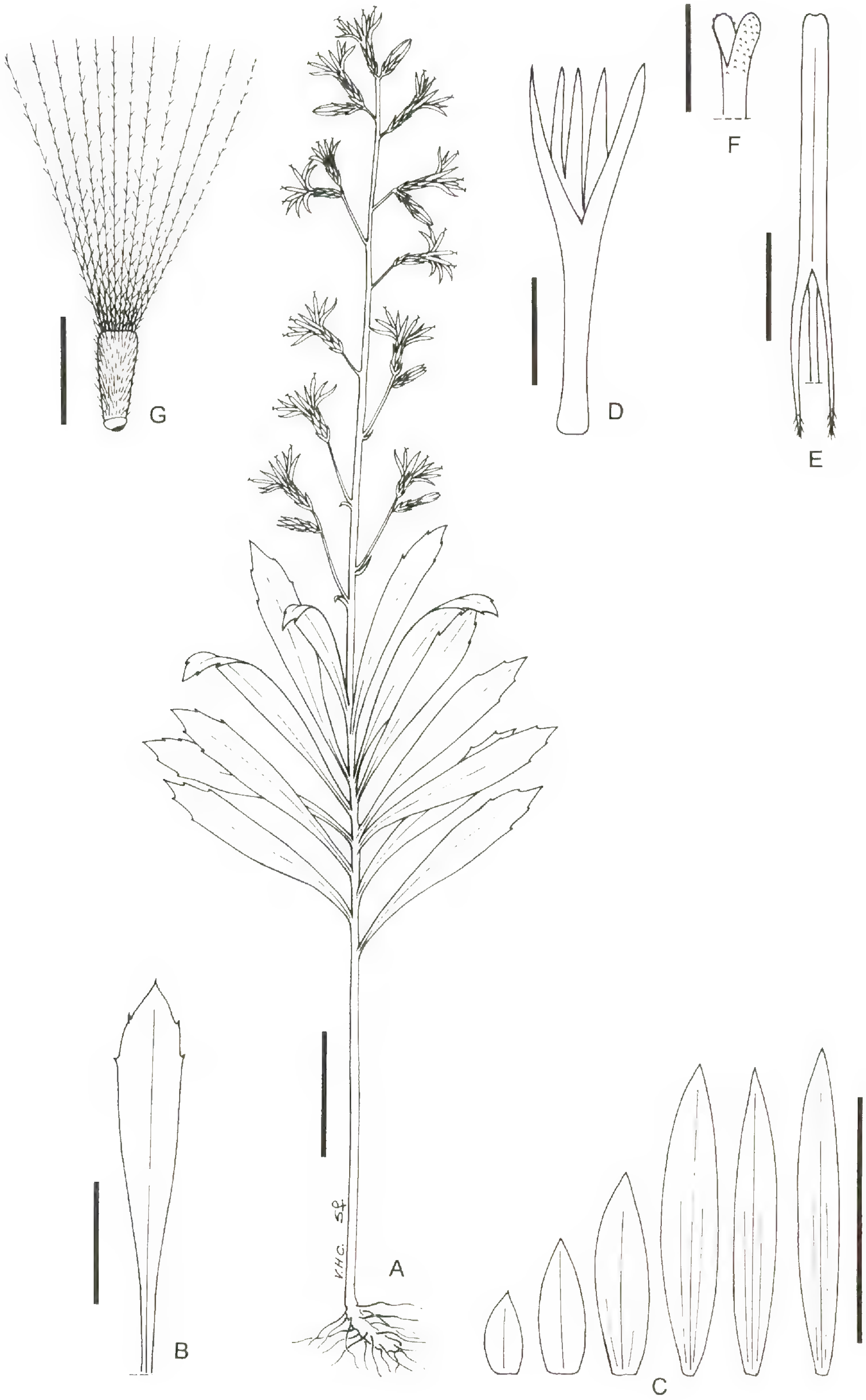

Figure 57. *Ainsliaea walkeri* Hook. f. —A. Habit. —B. Leaf. —C. Phyllaries. —D–G. Chasmogamous floret: D, corolla; E, stamen; F, style branches; G, achene with pappus. Scale bars: A = 3 cm; B = 2 mm; C = 6 mm; D, G = 2 mm; E, F = 1 mm. Drawn from *Tsang 24542* (MO).

glabrous, 0.5–1.3 cm; leaf blades chartaceous, linear to elliptic, (2.5)3.5–5.5 × 0.4–0.9 cm, gradually attenuated into the petiole, acute and mucronate at the apex, margins slightly revolute, with 1 or 2 mucronate teeth on each side toward the apex; slightly discolorous, glabrous on both surfaces; pinnate, apparently 1-nerved. Capitula short-pedunculate to subsessile, arranged in thyrsoid inflorescence; peduncles sparsely bracteolate, with upper bracteoles transitioning to the involucre, 1–3 mm; involucre 6- or 7-seriate, cylindrical, 7–10 × 3–3.5 mm; phyllaries papyraceous, glabrous; outer phyllaries ovate, acute, 2–3 × 0.8–1.3 mm; inner phyllaries linear to elliptic, acute, 3- to 5-nerved, 6.5–8 × 0.7–1 mm. Florets (2)3, hermaphrodite, chasmogamous; corolla white, fragrant, ligulate, 4.5–7.5 mm, lobes ca. 1.5 mm (deeper slit ca. 3), tube 1.5–4 mm; anthers purple, 4–5 mm, apical appendages emarginate, ca. 0.5 mm; tails 1–1.5 mm, shortly pilose; style branches truncate, 0.4–0.5 mm. Achenes oblong, 1–2 mm, densely pilose; pappus bristles ca. 34, ca. 6 mm.

Distribution and phenology. China (Guangdong, Guangxi), collected on shaded wet cliffs and swampy thickets at ca. 600 m; flowering in October and December.

Relationships. *Ainsliaea walkeri* resembles *A. trinervis* in habit. Both species are from China, have verticillate leaves, and narrow leaf blades. *Ainsliaea walkeri* may be distinguished by its leaf margins with only one or two mucronate teeth on each side above (vs. uniformly denticulate).

Representative specimens examined. CHINA. **Guangxi:** Sch-feng, Dar Shan, S Nanning, *R. C. Ching 8158* (NY, W), *R. C. Ching 8246* (NY, UC); Kwangtung border, Shang-sze district, SE of Shang-sze, Shap Man Taai Shan, Tang Lung Village, *W. T. Tsang 24495* (MO, NY), Nam She Village, *W. T. Tsang 24542* (MO, NY, US), *W. T. Tsang 24807* (MO, NY).

NAMES OF DUBIOUS IDENTITY

Ainsliaea asperrima (D. Don) Sch. Bip., Jahresber. Pollichia 20–21: 407. 1863. Basionym: *Hieracium asperrimum* D. Don, Pr. Fl. Nepal.: 165. 1825. TYPE: Nepal. *Wallich s.n.* (K, not seen).

The protologue of *Hieracium asperrimum* D. Don includes the collection "Nepal, *Wallich s.n.*" without any reference to flower characters. I was unable to find any material of this exsiccata either in K, where Wallich collections are deposited, or in BM, BR, E, G, GH, L, NY, or S, where duplicates are probably kept. I found a specimen, "India, Khasia, Griffith s.n., distr. by Lemann 274," at P, wrongly marked as the isotype of *Ainsliaea asperrima* (D. Don) Sch. Bip. This collection was cited by Schultz Bipontinus (1863: 407) when he transferred *H. asperrimum* to the genus *Ainsliaea*. Although this collection seems to correspond to *A. asperrima* according to the original diagnosis (i.e., "foliis lanceolatis ... sinuoso-dentatis ..."), it lacks capitula and cannot be positively identified as a species of *Ainsliaea*. For this reason, until the type of Don's name is located or materials can be provided, the identity of *A. asperrima* is uncertain.

Ainsliaea × *hybrida* Sugimoto, J. Jap. Bot. 32(2): 63. 1957. TYPE: Japan. Kiushu, Insl. Yakushima, 13 Aug. 1956, *J. Sugimoto s.n.* (TI, not seen)

Ainsliaea mollis Diels ex H. Limpr., in Limpr., Feddes Repert. Beih. 12: 514. 1922. TYPE: China. Sichuan: "Szechuan," Lu ting tschian am Tung ho, zwischen Gan gou und Wu li gou, 2000 m, *H. Limpricht 1613* (B, not seen).

The name *Ainsliaea mollis* was mentioned by Tseng (1996) in *Flora Republicae Popularis Sinicae*. However, because I was unable to see the type specimen and I have seen no other material which can be assigned to this species, the identity of *A. mollis* is not clear.

NAMES ADDED IN PROOF

The following eight names in *Ainsliaea* were reported by Chuang (H. Chuang *in* H. Peng. 2004. Addenda. Fl. Yunnan. 13: 833–835) in treating *Ainsliaea* for the *Flora Yunnanica*. I have not yet seen the type specimens for these names and their taxonomic identity is not confirmed.

Ainsliaea angustifolia Hook. f. et Thoms. ex C. B. Clarke var. *luchunensis* H. Chuang

Ainsliaea fulvioides H. Chuang

Ainsliaea fulvioides H. Chuang var. *glabriachenia* H. Chuang

Ainsliaea gongshanensis H. Chuang

Ainsliaea henryi Diels var. *daguanensis* H. Chuang

Ainsliaea latifolia (D. Don) Sch. Bip. var. *ramifera* H. Chuang

Ainsliaea lijiangensis H. Chuang

Ainsliaea pingbianensis Y. C. Tseng var. *malipoensis* H. Chuang

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Appendix 1

List of accepted species of *Ainsliaea*. Numbering of species applies to Appendices 1 and 2 only.

1. *Ainsliaea acerifolia* Sch. Bip.
   1a. *A. acerifolia* var. *acerifolia*
   1b. *A. acerifolia* var. *subapoda* Nakai
2. *A. angustata* C. C. Chang
3. *A. apiculata* Sch. Bip.
   3a. *A. apiculata* var. *apiculata*
   3b. *A. apiculata* var. *acerifolia* Masam.
   3c. *A. apiculata* var. *ovatifolia* Masam.
4. *A. aptera* DC.
5. *A. apteroides* (C. C. Chang) Y. C. Tseng
6. *A. bonatii* Beauverd
   6a. *A. bonatii* var. *bonatii*
   6b. *A. bonatii* var. *multibracteata* (Mattf.) S. E. Freire
7. *A. brandisiana* Kurz
8. *A. caesia* Hand.-Mazz.
9. *A. cavaleriei* H. Lév.
10. *A. chapaensis* Merr.
11. *A. cordifolia* Franch. & Sav.
12. *A. crassifolia* C. C. Chang
13. *A. dissecta* Franch. & Sav.
14. *A. elegans* Hemsl.
    14a. *A. elegans* var. *elegans*
    14b. *A. elegans* var. *strigosa* Mattf.
15. *A. foliosa* Hand.-Mazz.
16. *A. fragrans* Champ. ex Benth.
17. *A. fulvipes* Jeffrey & W. W. Sm.
18. *A. glabra* Hemsl.
    18a. *A. glabra* var. *glabra*
    18b. *A. glabra* var. *sutchuenensis* (Franch.) S. E. Freire
19. *A. glumacea* (Fr.) Sch. Bip.
20. *A. gracilis* Franch.
21. *A. grossedentata* Franch.

22. *A. henryi* Diels
    22a. *A. henryi* var. *henryi*
    22b. *A. henryi* var. *subalpina* (Hand.-Mazz.) S. E. Freire
23. *A. hypoleuca* Diels ex H. Limpr.
24. *A. kawakamii* Hayata
25. *A. lancangensis* Y. Y. Qian
26. *A. latifolia* (D. Don) Sch. Bip.
    26a. *A. latifolia* var. *latifolia*
    26b. *A. latifolia* var. *taiwanensis* S. E. Freire
27. *A. linearis* Makino
28. *A. macrocephala* (Mattf.) Y. C. Tseng
29. *A. macroclinidioides* Hayata
30. *A. mairei* H. Lév.
31. *A. nana* Y. C. Tseng
32. *A. nervosa* Franch.
33. *A. oblonga* Koidz.
    33a. *A. oblonga* var. *oblonga*
    33b. *A. oblonga* var. *latifolia* Kitam.
34. *A. parvifolia* Merr.
35. *A. paucicapitata* Hayata
36. *A. pentaflora* S. E. Freire
37. *A. pertyoides* Franch.
    37a. *A. pertyoides* var. *pertyoides*
    37b. *A. pertyoides* var. *albotomentosa* Beauverd
38. *A. pingbianensis* Y. C. Tseng
39. *A. qianiana* S. E. Freire
40. *A. ramosa* Hemsl.
41. *A. reflexa* Merr.
42. *A. rubrinervis* C. C. Chang
43. *A. smithii* Mattf.
44. *A. spanocephala* Y. C. Tseng
45. *A. spicata* Vaniot
46. *A. trinervis* Y. C. Tseng
47. *A. uniflora* Sch. Bip.
48. *A. walkeri* Hook. f.
49. *A. yunnanensis* Franch.

Appendix 2

List of exsiccatae. The number in parentheses refers to the species number in Appendix 1. When collections were made by more than one collector and the numbering system of one collector is used, only that collector is cited.

Amano 6874 (29), 6903 (29), 7485 (29); Anders 11129 (4); Arimoto in 1903 (3a); Averyanov et al. N VII471 (41).

Bangham W. N. & C. M. 1191 (41); Bartlett 6215 (22b); Bartholomew 1532 (4); Bartholomew & Boufford 3878 (4); Bartholomew et al. 1598 (23), 1970 (20), 2041 (20); Bay s.n. (3a); Beusekon & Phengklai 2370 (26a) [type, *A. pteropoda*]; Biskam 43 (26a); Biswas 3508 (4), in 1935 (5); Bock & Rosthorn 773 (22a) [type, *A. undulata*], 877 (21) [type, *A. gracilis* var. *robusta*], 1220 (6a) [type, *A. multibracteata*]; Bodinier 79 (37a) [type, *A. sparsiflora*]; Bodinier 1584 (45); Bodinier & Deblenne 78 (37b) [type, *A. ovalifolia*]; Bodinier & Ducloux 8 (49), in 1897 (23); Boufford & Kato 23362 (1b); Boufford & Mitsuta 20043 (3b), 20062 (27); Boufford & Shimizu 23468 (1b); Boufford & Wood 19871 (1b); Bunchuai 1437 (26a), 1501 (44); Burger s.n. (47); Burger & Zollinger 266 (3a) [type, *A. apiculata*], 272 (1a) [type, *A. acerifolia*], 283 (17) [type, *A. uniflora*].

Cavalerie 19 (37b), 20 (30), 21 (45), 22 (49), 1907 (9) [type, *A. cavaleriei*], 4212 (37b), 7485 (37b); Champion 161 (16) [type, *A. fragrans*]; Chang 0694 (6a); Charette 1525 (3a), 1897 (1b); Chen B. et al. 80 (16); Chen D. 425 (9); Chen H. 2190 (16); Chen N.G. 42854 (16); Chen S. Q. 2693 (24), 14753 (9), 15094 (38), 15486 (17), 6610 (46) [type, *A. trinervis*], 17037 (46), 17243 (9); China Germany Team 1024 (16); Ching 2459 (24), 3861 (24) [type, *A. hui*], 4133 (24), 4463 (16), 8158 (48), 8246 (48); Chiou 042 (37b), 59608 (26a), 60097 (49); Chong 3882 (16); Chow C. L. 1909 (16); Chow H. C. 1978 (16); Chu 2273 (2), 2564 (23), 2564 (23), 3939 (18a); Chuang & Kao 2474 (26b), 2526 p.p. (22b, 41), 4198 (41); Chung B. C. in 1960 (1b); Chung H. H. 2906 (16); Chung I. C. 7080 (1b); Clarke 37392 (45); Clemens 16277 (41), 17256b (41), 18906 (41), in 1925 (41), in 1928 (41); Clifford G. Rice 10-78 (26a); Conover 2830 (29); Cui, S. C. 314 (1b).

David in 1869 (18a) [type, *A. lancifolia*]; Dayao S. Team 12856 (20); de Wilde W. J. J. O. & Wilde-Duyfjes B. E. E. 14245 (41), 15196 (41), 16734 (41); Delavay 607 (6a) [type, *A. pteropoda* var. *platyphylla*], 608 (49) [type, *A. yunnanensis* and *A. pteropoda* var. *leiophylla*], 1218 (37a) [type, *A. pertyoides*], 1921A (45) [type, *A. pteropoda* var. *obovata*], 2313 (37a) [type, *A. pertyoides*], 4939 (32) [type, *A. nervosa*], in 1888 (37b), 12 Jan. 1889 (37b), 28 Jan. 1889 (37a, 37b), Oct. 1889 (49), in 1895 (49); Deng 347 (16), 1449 (24), 6306 (24), 7048 (16), 7582 (46) [type, *A. trinervis*], 8023 (9), 8530 (16), 8878 (9); Dillon Ripley & Ulmer 43 (41), 71 (41); Ding & Shi 1087 (24); Drummond 1846 (4), 20055 (26a), 22635 (4), 25596 (26a); Ducloux 9D (45) [type, *A. spicata*], 253 (6a) [type, *A. bonatii* var. *arachnoidea*], 254 (37b) [type, *A. pertyoides* var. *albotomentosa*], 256 (37a), 295 (37b), 296 (37b) [type, *A. pertyoides* var. *intermedia*], 2271 (37b), 2348 (37b), 2118 (37b), 3646 (6a) [type, *A. bonatii*], 6028 (37b); Dudgeon & Kenoyer 36 (26a); Duthie 12985 (26a), 1739 (4), 19884 (4), 25653 (4).

Ecker 131 (4); Elliott 1104 (33a); Elmer 8363 (41), 14193 (41), 14293 (41); Esquirol 3633 (14a) [type, *A. elegans* var. *tomentosa* and *Pertya esquirolii*], in 1917 (23); Exped. Biolog. Sino-Rossica in 1955 (26b).

Faber 128 (32), 391 (18a) [type, *A. glabra*], in 1885/86 (16); Fan & Li 620 (16), 621 (20); Fang W. P. 1990 (18a), 1996 (18a), 2076 (42) [type, *A. rubrinerris*], 3157 (18a); Fang W. Z. 8017 (16); Fang (24), 39 (24); Farges 333 (21) [type, *A. grossedentata*], 1034 bis (16) [type, *A. rubrifolia*], 1034A (20) [type, *A. gracilis*], 1034B (18b) [type, *A. sutchuenensis*], s.n. (18b, 21); Faurie 409 (1b), 1918 (1b), 1920 (1b), 4065 (27) [type, *A. faurieana*], 4066 (3b) [type, *A. liukiuensis*], 4220 (3a), 5451 (1b), 6079 (3a), 6356 (1b), 6611 (3a), 6740 (1b), 11716 (17), in 1900 (3b); Feng K. M. 12964 (14a); Feng Z. 600 (16); Ford s.n. (16); Forrest 5681 (22b) [type, *A. henryi* var. *oratifolia*], 6545 (28) [type, *A. pteropoda* var. *macrocephala*], 7862 (17) [type, *A. fulvipes*], 7865 (45), 11070 (12) [type, *A. crassifolia*]; Foxworthy 2400 (41); Fukasawa in 1910 (16); Furuse 28 Sep. 1955 (1b), 29 Sep. 1955 (13), Nov. 1955 p.p. (3a, 3b), July 1956 (1b), Sep. 1956 (1b), 7 Oct. 1956 (11) [type, *A. cordifolia*], 9 Oct. 1956 (47), Sep. 1957 (1b), 27 Oct. 1957 (3a), 28 Oct. 1957 (13), Sep. 1958 (1b), 24 Oct. 1958 (1b, 3a), Nov. 1958 (11), in 1959 (1b), in 1960 (3a), 22 Sep. 1961 (1b), 30 Sep. 1961 (3a), Oct. 1961 (47), in 1961 (3a).

Gao 50833 (16), 51997 (16), 53649 (16), 53659 (24); Garrett 618 (26a), 643 (26b); Gates et al. 7262 (41); Geesink et al. 8170 (26a); Griffith 3329/1 (19), 3330/1 (4), 3331 p.p. (26a, 45); Guangdong 77 Team 5544 (16), 5555 (38).

Hagerup in 1916/17 (41); Hamel & Si Toroes Rahmat 615 (41) [type, *A. longipetiolata*]; Hance 240 (16); Hancock 8 (49) [type, *A. scabrida*]; Handel-Mazzetti 293 (45), 511 (37a), 582 (37b), 864 (45), 1835 (23), 2485 (30), 2615 (23), 3282 (22b) [type, *A. reflexa* var. *subalpina*], 3619 (28), 5383 (39), 5683 (30), 6255 (45), 7623 (26a), 7808 (22b) [type, *A. reflexa* var. *subalpina*], 8343 (22b) [type, *A. reflexa* var. *nimborum*], 8501 (26a); Hatusima 16515 (16); Hennipman 3608 (44) [type, *A. spanocephala*]; He 4125 (24); Henry 1422 (40) [type, *A. ramosa*], 6637A (21), 6639 (22a) [type, *A. henryi*],

7461 (22a), 7599 (18b) [type, *A. tenuicaulis*], 9108 (14a) [type, *A. elegans*], 9294 (45), 9294 A (45), 9294 B (45), 9851 (49) [type, *A. scabrida*], 9882A (37a), 9882B (37a), 9907 (5), 9907A (5) [type, *A. pteropoda* var. *apteroides*], 9926 (37b), 9955 (6a), 10891 (14b) [type, *A. elegans* var. *strigosa*], 12763 (26a), 12763A (45), 12763B (26a), in 1892 (16); Hiroe 1167 (1b), 12044 (3a); Ho S. Y. 7190 (24); Ho H. L. et al. 611 (41), 879 (41); Hooker s.n. (19); Hooker & Thompson s.n. in Kashmir (4), in Khasia, 4000–6000 ft. (19) [type, *A. angustifolia*], in Khasia 4000–5000 ft. (4), in Khasia 5000–7000 ft. (26a, 45), in N Himalayas (4), in Nepalia-Kamaon (26a) [type, *A. pteropoda*], in Sikkim 5000–7000 ft. (4), in Sikkim 6000–8000 ft. (26a) [type, *A. pteropoda var. effusa*]; Hosseus 358a (26b); Hou 74236 (49), 74248 (23), 74327 (5); Hsieh & Hsiao in 1988 (29); Huang M. 111593 (16); Huang S. F. 2588 (29), 4990 (26b); Huang T. C. et al. 5798 (22a); Hügel 719b (4), 1241 (4); Hulten in 1961 (1b).

Ichikawa 128 (3a); Iketsu et al. 1099 (1b); Im & Kawahara 7815 (3a); Inoue et al. 1760 (1b); Iwatsuki F. & Fukuoka T3558 (45); Iwatsuki K. et al. 15 (29), 113 (27), 517 (29), 556 (29).

Jacobs 7081 (41); Jackemont 490 (26a); Jin 382 (24); Jisiba in 1926 (1b), in Feb. 1927 (3a), in Sep. 1927 (1b); Jutila & Fujino 751 (1b).

Kamini in 1961 (4); Kanai 655 (3a); Kanashiro 23 (29), 2004 (29); Kanehira 3051 (22b) [topotype, *A. elegans* Hayata], 21194 (22b), 2945 (22b); Kanehira & Sasaki 21644 (41), 21765 (29); Kapoort 75623 (45); Kato et al. 151 (29); Kawagoe in 1917 (3a), in 1919 (29); Kazmi in 1965 (4); Keng H. K1214 (41); Keng Y. L. 1019 (24) [type, *A. hui*]; Kingdon Ward 447 (26a), 17086 (26a), 18260 (26a); Kings in 1897 (19); Kirat in 1926 (26a), in 1932 (26a); Kirino 356 (3a), 392 (1b); Kitamura in 1932 (3b); Kobayashi 12864 (1b), 12921 (1b), 12982 (3a), 13627 (1b), 14195 (1b), 15072 (3a), 15169 (3a), 15174 (3a), 15175 (3a), 15188 (1b), 16547 (3a); Koelz 129 (4), 3168 (4), 1789 (4), 7410 (4), 10517 (26a), 20156? (4), 27006 (26a); Koerper s.n. (26b); Koidzumi in 1921 (3b, 3c), in 1923 (29); Kollhoff 218 (16); Konta & Wakabayashi 357 (13); Koyama 2678 (3a); Koyama & Phengklai T-39997 (26a); Koyama et al. 237 (29), T-30524 (26a), T-31605 (44), T-31642 (44), T-33435 (26b), T-33444 (26a), 33699 (44) [type, *A. spanocephala*]; Koyama 8643 (41); Kuntze 3280 (3a), 6695 (26a), 6956 (4) [type, *A. aptera* f. *paniculata*]; Kurosaki 5676 (29); Kurz 865 (7) [type, *A. brandisiana*].

Lau S. K. 4041 (33a), 4554 (9); Lau Y. K. 111 (16); Lee I. K. in 1957 (1b); Lee Y. N. & Lee Y. S. 709 (1b); Li M. K. 0724 (45); Li Z. 317 (24), 716 (16), 2031 (16); Li Z. Y. et al. 326 (22a); Liang (?not clear) B. Z. 83655 (16); Limpricht 1251 (23) [type, *A. hypoleuca*]; Lin & Yen 348 (41); Linsley Gressitt 195 (41); Liu S. L. 890169 (16); Liu X. 24979 (17), 25245 (24), 28990 (46); Liu Y. S. 1748 (16), 2325 (32); Liu Z. 28894 (24); Liu Z. Y. 15451 (18b); Lobb 447 (41) [type, *A. reflexa* var. *lobbiana*]; Loher 3621 (41), 6474 (41), 14045 (41); Lu & Wei 20097 (9).

Mackinnon s.n. (26a); Maire 102 (37b), 352 (37b), 919 (49), 920 (6a), 921 (30), 1084 (37b), 2349 (6a), 2401 (37b), 2517 (37b), 2529 (39) [type, *A. qianiana*], 2529 (49), 3155 (23), 3155B (23), 3225 (45), 3936 (30), 3917 (49), Nov. 1912 (30) [type, *A. mairei*], W7135 (45), W7224 (49), W7225 (18a), W7227 (26a), W7228 (45), W7229 (23), W7231 (23), W16887 (37b); Malaise 158 (26a), 186 (26a); Manberg 684 (22b) [type, *A. henryi* var. *ovatifolia*]; Maruo in 1913 (11) [type, *A. maruoi*]; Masamune in 1922 (3a), in 1927 (27); Maser 118 (4); Masumura in 1931 (22b); Mauri in 1921 (26a); Maximowicz 13147 (3a), in 1863 (3a), in 1863 (1a), in 1863 (47); McClure 459 (9) [type, *A. cleistogama*], 16069 (41); Mearns 19 (41), 20 (41); Meebold 838 (26a), 6805 (45); Mell 874 (8) [type, *A. caesia*], in 1914 (30); Merrill 734 (41), 835 (41), 4571 (41) [type, *A. reflexa*], 4824 (41), 10230 (16), 10237 (34) [type, *A. parvifolia*]; Miki et al. 56 (1b); Mimoro et al. 4117 (47), 4197 (47), 5454 (47); Mitsuta & Doei 411 (27); Miyagi in 1911 (29); Miyasato s.n. (29); Mizushima 12738 (1b), 13199 (3b); Mohindar Nath 11 (4); Moran 4722 (1b), 5322 (27), 5333 p.p. (3a, 3c); Muramatsu in 1935 (3b) [type, *A. apiculata* var. *acerifolia*]; Murata 10486 (23), 17066 (29), 41060 (13), 45392 (1b); Murata Koyama 41255 (1b); Murata & Shimizu 1530 (1b); Murata & Tabata 418 (3c) [type, *A. apiculata* var. *ovatifolia*]; Murata et al. 150 (1b), 15480 (27), 15716 (27), T-15998 (26b).

Nagasawa et al. in 1905 (22b) [type, *A. elegans* Hayata]; Naito in 1927 (29b); Narita in 1910 (16) [type, *A.cordifolia* var. *integrifolia*]; Nicolson 2955 (26a); Niyomdham & Vidal 438 (44).

Ohashi et al. 708054 (1b); Ohba in 1924 (29); Okada et al. 235 (29).

Panigrahi 4798 (19), 6184 (26a), 19832 (19); Parot 51434 (19); Pételot 417 (36) [type, *A. pentaflora*], 1452 (23), 1767 (41) [type, *A. tonkinensis*], 2067 (41), 2068 (10) [type, *A. chapaensis*], 2082 (23) [type, *A. petelotii*], 3680 (41), 3724 (14b) [type, *A. elegans* var. *strigosa*], 4551 (36), 4552 (23) [type, *A. petelotii*]; Prain 23896 (26a).

Qian 3343 (25) [type, *A. qianiana*]; Quisumbing & Sulit 6240 (41).

Raince & Kessliz in 1886 (1b); Raizada in 1937 (4); Ramos & Edaño 37614 (41), 40191 (41), 40469 (41), 44917 (41); Rock 4072 (37a), 4216 (41), 5628 (28) [type, *A. pteropoda* var. *macrocephala*], 5773 (28) [type, *A. pteropoda* var. *macrocephala*], 6679 (6a), 6937 (49), 7284 (17), 17427 (5); Royle in 1833 (4) [type, *A. aptera*], s.n. (26a) [type, *A. pteropoda*].

Saito et al. in 1975 (1b); Sakaguchi in 1922 (29); Sako 6216 (29); Santos 32047 (41); Sasaki in 1919 (3b); Sastry 41016 (19); Sata in 1932 (3b); Satomi in 1957 (11); Satomi 14467 (1b), 15301 (3a); Sawson 15337 (4); Schlagintweit Cat. N° 391, 1/30 Oct. 1855 (19) [type, *A. glumacea* Klatt], Cat. N° 10864, 15/18 Nov. 1856 (19), Cat. N° 11960, 10–14 Feb. 1857 (19); Schneider 2328 (28) [type, *A. pteropoda* var. *macrocephala*]; Seto 30054 (47); Sharp s.n. (4); Shi 15077 (9); Shimizu 13558 (1b), 20394 (22b); Shimizu & Kozima 33 (3b); Shoma & Takahashi in 1974 (1b); Shoma et al. in 1970 (47); Si Boeea Rahmat 10132 (41), 10451 (41); Simond 30405 (26a); Sin 181 (18b) [type, *A. plantaginifolia*]; Singh 12786 (26a); Singh & Jalan in 1960 (4); Si Toroes Rahmat 615 (41) [type, *A. longipetiolata*]; Smith 4698 (43) [type, *A. smithii*], 4745 (6b) [type, *A. mattfeldiana*], 12120 (15) [type, *A. foliosa*]; Smitinand in 1982 (44); Smitinand & Anderson 7323 (26a); Smitinand & Alsterlund 6695 (26a); Smitinand et al. 10299 (26a); Sonohara s.n. (29), 642 (29), in 1922 (29); Sonohara et al. 6286 (29); Steward A. N. 1292 (24); Steward A. N. & Cheo 1090 (9); Steward A. N. et al. 418 (21); Stewart R. R. 5203 (4), 6836 (4), 13506 (4), 10100B (4), 15787 (26a), 17166 (4), 27535 (4), in 1978 (4); Stolitzka in 1864 (4a, 26a); Sugaya et al. in 1952 (1b); Sugimoto 10912 (13) [type, *A. dissecta*]; Sun 1462 (18b); Suzuki 93 (3a), 107 (1b), 141 (3a), 145 (1b), 549 (26b), 1450 (41), 2256 (41), 9513 (26b), in 1928 (22b), July 1929 (41), Aug. 1929 (22b); Suzuki & Kudo in 1928 (41).

Taam 113 (9); Tagawa 557 (1b); Tagawa & Iwatsuki 4655 (29); Tagawa & Motozi 1921 (3b), 1970 (27); Tagawa et al. 1611 (45), T-2866 (26b); Takaata & Kobayashi 575 (1b); Takahashi 1838 (1b); Takaki in 1957 (3a); Takeuchi M. & T. 14 (1b); Tamura 9327 (3a), 21079 (22b); Tamura & Koyama 23261 (22b), 23388 (26b), 23595

(22b), 23729 (26b), 23780 (26b); Tan 59543 (16), 59820 (20), 59863 (16), 60508 (16), 62920 (22a); Tanaka T. 10392 (3b); Tanaka Y. 46 (3a); Tang 5788 (16); Taquet 243 (3a), 1062 (3a); Tashiro in 1910 (16), 6 Aug. 1918 p.p. (3b, 3c), 9 Aug. 1918 p.p. (3a, 3b), in 1923 (29), in 1924 (29); Tawada 29 (29), 39 (29); Tateishi & Murata 4770 (29c); Ten 354 (18a); Teraya 459 p.p. (22b, 41); Togasi 1448 (27) [type, *A. linearis*]; Tsang 20084 (40), 20812 (16), 24495 (48), 24542 (48), 24807 (48), 28488 (20), 28500 (22a); Tsang & Wong 3177-herb 15038 (16); Tsiang 5087 (18a), 7913 (20); Tsugaru et al. 7348 (1b).

Uchida in 1969 (11); Uno 23338 (1b); Utech 98-415 (1b).

Vera Santos 5436 (41).

Wagner 6515 (22b), 6516 (26b) [type, *A. latifolia* var. *taiwanensis*]; Walker 7631 (29c); Wallich C-44 (26a), in 1821 (26a); Wand & Zhang 8241 (9); Wang C. 35545 (41), 35546 (41), 40279 (40), 40798 (40), 42244 (24), 44058 (16), 44094 (16); Wang C. W. 82572 (14a); Wang F. T. 22682 (32); Wang Q. W. 83962 (23); Wang W. H. 8078 (18a); Watabiki in 1902 (3a); Wawra 1193 (3a); Wei F. 230 (9), 1453 (20); Wei Z. 120645 (24); Weigold in 1915 (23); Wilkes in 1875 (48) [type, *A. walkeri*]; Williams 1434 (41); Wilson E. A. 183 (6a); Wilson E. H. 1766(18b) [type, *A. tenuicaulis*], 1811 (20), 2159 (22a), 2743 (21); Winit 1344 (26a); Wissmann 184 (49), 230 (49), 411 (49), 425 (6a), 456 (14a), 777 (26a) [type, *A. heterantha*]; Wuyi S. Team 80-0203 (22a).

Yamaguchi & Nagano in 1917 (3a, 3b); Yamazaki 3103 (47); Yang K. C. 1318 (41); Yang H. N. 2039 (3b); Yang K. C. 791 (24); Yoa et al. 79344 (24).

Zhang Y. B. 0283 (17); Zhang Z. 13248 (38).

Appendix 3

List of voucher specimens used for outgroup taxa in cladistic analysis.

Macroclinidium robustum Maxim. JAPAN. Yokohama: *Dr. Wawra 1527* (NY).

Myripnois dioica Bunge. CHINA. Peking vic.: *F. T. Wang 20106* (NY).

Pertya ovata Maxim. JAPAN. Honshu. Wakayama: *Takahashi et al. 1387* (MO). Pref. Kyoto, near Yamashirotoge, *G. Murata 19177* (NY).

Appendix 4

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CARACTERES DIAGNÓSTICOS FOLIARES EN ESPECIES NEOTROPICALES DE *MARATTIA* SW. (MARATTIACEAE–PTERIDOPHYTA)[1]

María del Carmen Lavalle[2]

Resumen

Se estudiaron comparativamente distintos caracteres de la morfología foliar en las siete especies neotropicales del género *Marattia* Sw.: *M. alata* Sw., *M. cicutifolia* Kaulf., *M. excavata* Underw., *M. interposita* Christ, *M. laevis* Sm., *M. laxa* Kunze y *M. weinmanniifolia* Liebm. con el fin de analizar su valor diagnóstico. Los resultados obtenidos se aplican en una clave para la determinación de las especies.

Abstract

This is a study of the comparative foliar morphology carried out in seven neotropical species of the genus *Marattia* Sw.: *M. alata* Sw., *M. cicutifolia* Kaulf., *M. excavata* Underw., *M. interposita* Christ, *M. laevis* Sm., *M. laxa* Kunze, and *M. weinmanniifolia* Liebm., with the purpose of analyzing their diagnostic characters. A key to identify the species is obtained from the results.

Key words: epidermis, indument, *Marattia*, Marattiales, morphology, Neotropical.

El número de familias y géneros incluidos en Marattiales ha variado de acuerdo con los distintos autores y en el transcurso del tiempo (Christensen, 1938; Campbell, 1940; Copeland, 1947; Pichi Sermolli, 1977; Hill & Camus, 1986). En este trabajo se sigue a Rolleri et al. (2003), quienes luego de analizar el valor diagnóstico de numerosos caracteres vegetativos y esporales, adoptaron una sola familia, Marattiaceae Berchtold & C. Presl formada por 5 géneros vivientes. Tres de ellos son paleotropicales: *Angiopteris* Hoffmann, con unas 20 especies (Rolleri, 2002). *Archangiopteris* Christ & Giesenhagen, con 6 a 8 especies (Mengascini, 2002) y *Christensenia* Maxon, con 2 especies y 2 subespecies (Rolleri, 1993); uno es neotropical, *Danaea* J. E. Smith, con 17 especies (Rolleri, 2004); y un género es pantropical, *Marattia* Sw. En relación con *Marattia*, Lavalle (2003) efectuó la primera revisión del género para el neotrópico, delimitando 7 especies: *M. alata* Sw., *M. cicutifolia* Kaulf., *M. excavata* Underw., *M. interposita* Christ, *M. laevis* Sm., *M. laxa* Kunze y *M. weinmanniifolia* Liebm. Si bien no existen revisiones de las especies del paleotrópico, en algunos trabajos recientes se sugiere que habría poco más de 10 (Lavalle, 2003; Rolleri et al., 2003). Rolleri et al. (2001a) analizaron diferentes caracteres foliares en cinco especies paleotropicales y posteriormente Lavalle y Stampella (2004) completaron ese estudio con uno similar de especies endémicas de Hawaii, Nueva Caledonia y Nueva Guinea.

Tanto la familia Marattiaceae como los géneros en ella incluidos carecieron por mucho tiempo de estudios de conjunto prevaleciendo el tratamiento de las especies en floras (Underwood, 1909; Domin, 1929; Selnem, 1967; Stolze, 1976; Proctor, 1985, 1989; Mickel & Beitel, 1988; Lellinger, 1989; Tryon & Stolze, 1989; Camus & Pérez García, 1995; Smith, 1995; Tuomisto & Moran, 2001). Pero también hay trabajos de estudios morfológicos en los que se amplía el conocimiento de las especies con el análisis de los modelos epidérmicos y el indumento (Hill & Camus, 1986; Rolleri et al., 1987, 1991, 2003; Rolleri, 1993, 2002, 2004; Mengascini, 2002), los estomas maratiáceos (Rolleri & Lavalle, 1997) y la naturaleza silícea de los idioblastos epidérmicos (Rolleri et al., 2001b).

De acuerdo a lo presentado por Lavalle (2005) las plantas de *Marattia* tienen esporófitos grandes, de hasta 4–5 m de altura, con rizomas desnudos, subterráneos y erguidos; frondas con gruesos estípites que representan aproximadamente la mitad de la altura total de las frondas; láminas deltoides, monomórficas, 2-pinnadas en *M. cicutifolia* y *M. laxa*, 3–4-pinnadas en el resto de las especies. Las venas son libres, exclusivamente simples, exclusivamente bifur-

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cadas o combinaciones de venas simples con bifurcadas o bifurcadas con geminadas. Las estructuras esporógenas están formadas por sinangios intramarginales a supramediales que producen numerosas esporas monoletes.

En este trabajo se han analizado varios caracteres del esporófito de las especies neotropicales de *Marattia*, con el fin de evaluar su implicancia taxonómica y su valor diagnóstico en la delimitación de las especies. Se tomó la epidermis de las frondas como punto de partida de comparación, ya que ofrece en Marattiaceae rasgos diagnósticos estables en el nivel específico (Rolleri et al., 2003). Estos caracteres podrían relacionarse con otros rasgos de apreciación más sencilla, como los caracteres externos de la lámina, cuya utilización permita una determinación más confiable de las especies.

Aquí se llevó a cabo un estudio de morfología, naturaleza y distribución del indumento de láminas y ejes; epidermis adulta de hipofilos y epifilos; tamaño, densidad y morfología de los estomas adultos; y presencia, agrupación y localización de los idioblastos. Los resultados se compararon con datos previamente conocidos de las especies paleotropicales del género y de otros géneros de Marattiaceae y se aplicaron en la elaboración de una clave para la determinación de las especies.

Materiales y Métodos

materiales

Se utilizó material de los siguientes herbarios: CR, F, FI, GH, IJ, LP, MICH, MO, NY, RB, SI y US, citados de acuerdo con Holmgren et al. (1990). Aquí sólo se cita el correspondiente a las ilustraciones, las citas completas pueden consultarse en Lavalle (2003).

Marattia alata Sw. CUBA. **Oriente:** Crest of Sierra Maestra between Pico Turquino and La Bayamesa, *Morton & Acuna 3760* (GH, NY). JAMAICA. **Morce's Gap:** *Harris 7217* (F, IJ, NY). Blue Mt. Range: windward side of Morce's Gap, *Chrysler 1706* (MICH, NY).

Marattia cicutifolia Kaulf. BRASIL. **Minas Gerais:** Saramentra, Ouro Preto, *Glaziou 20987* (US). **Paraná:** Ipiranga, Serra do Mar, *Dusén 15417* (F, GH, MO, US). **Río de Janeiro:** Tijuca, *Brade 12554* (LP). **Santa Catarina:** Sertaô do Lagoa, *Rohr 1036* (SI, US). **Sâo Paulo:** Serra da Bocaina, *Luederwaldt 20976* (NY).

Marattia excavata Underw. EL SALVADOR. **Chalatenango:** Cerro El Pital, *Seiler 436* (F). GUATEMALA. **Alta Verapaz:** W of San Julián, *Williams et al. 40406* (F). HONDURAS. **Intibucá:** Cordillera Opalaca, bosque nebuloso y húmedo El Duraznillo, *Molina & Molina 14097* (F, NY). NICARAGUA. **Granada:** Volcán Mombacho, east side, near summit above Finca Las Delicias, *Bruce Nelson 7545* (GH).

Marattia interposita Christ. COSTA RICA. **Limón:** Cordillera de Talamanca, Cerro Bitarkara, Reserva Biol. Hitoy Cerece, *Herrera 2489* (CR). GUATEMALA. **Quetzaltenango:** Fuentes Georginas, western slope of Volcán de Zunil, *Standley 67512* (F). PANAMÁ. **Chiriquí:** Humid forest around Los Siguas Camp, *Maxon 5424* (NY, US): Boquete, Bajo Chorro, *Davidson 264* (F).

Marattia laevis Sm. ARGENTINA. **Misiones:** General Belgrano, ruta Nac. 101, Salto Andresito, *Guaglianone et al. 2975* (SI). BRASIL. **Río de Janeiro:** Guanabara, Tijuca, Estrada Vista Chinesa, *Sobrinho 1619* (LP). COLOMBIA. **Cundinamarca:** Sibaté, bosques de San Miguel, *Murillo 437* (F, NY). COSTA RICA. **Heredia:** Volcán Barba, S of the principal peak above Porrosatí, *Lellinger 1709* (F, US). ECUADOR. **Carchi:** Cerro Golondrinas, upper Rio Gualpí watershed, *Boyle & Hibbs 2354* (MO). PERÚ. **Cuzco:** La Convención, *Dudley 10619* (GH, MO). VENEZUELA. **Trujillo:** Boconó, 22 km SE of Boconó, rd. to Guaramacal, *Smith 1505* (MO).

Marattia laxa Kunze. HONDURAS. **Comayagua:** El Portillo, Montaña Comayagua, *Hazlett 2493* (F). MÉXICO. **Veracruz:** Xico, pequeña cañada entre Tonalaco y Oxtlapa, *Cházaro & Hernández 4037* (F, GH, MICH, US). PANAMÁ. **Chiriquí:** Southern slopes of Cerro Horqueta N of Boquete, *Wilbur et al. 13455* (GH, MICH).

Marattia weinmanniifolia Liebm. MÉXICO. **Chiapas:** Tenejapa, Banabil, *Breedlove 21993* (F, MICH, NY). **Jalisco:** Steep mountains 11–12 mi. S of Talpa de Allende, in the headwaters of a west branch of Rio de Talpa, *McVaugh 20393 y 21417* (ambos MO y NY). **Oaxaca:** Totontepec, Mixe, atrás de El Cerro de La Mitra, a 6 km al O de Totontepec, *Ramírez & Ramírez 100* (GH).

métodos

Para analizar el indumento se ablandaron los ejes y láminas con una solución de éter monobutílico del etilenglicol diluida 1:1 en agua.

Para estudiar los modelos epidérmicos, estomas e idioblastos se rehidrataron trozos de lámina, se aclararon con hidróxido de sodio 5% acuoso, y se blanquearon con hipoclorito de sodio comercial diluido 1:1 en agua. Luego se colorearon con safranina–fast green 2% en éter monometílico del etilenglicol según Gurr (1965: 308) y las muestras se montaron en "Canadax Biopur", un medio de montaje artificial estéril.

La densidad de los idioblastos se midió en número de idioblastos/mm^2 de superficie foliar; se consideró, arbitrariamente: densidad baja: 0–10 idioblastos/mm^2; media: 11–20 idioblastos/mm^2; y alta: 21–35 idioblastos/mm^2.

La terminología usada para describir las células epidérmicas es la propuesta por Rolleri y Deferrari (1986); la utilizada para el estoma maratiáceo, de Rolleri y Lavalle (1997); y la aplicada a los estomas adultos, de Wilkinson (1979). Todos los términos pteridológicos están definidos en Lellinger (2002).

Resultados y Discusión

indumento (fig. 1)

El indumento se utilizó como un carácter sistemático en las floras regionales del neotrópico. En

Marattia el indumento es escaso y está representado por dos tipos morfológicos básicos: tricomas (Lellinger, 2002) y protoscamas. Aquí se considera protoscamas a las enaciones epidérmicas paucicelulares o pluricelulares aplanadas, a menudo con lobulaciones irregulares o piliformes. La mayoría de las protoscamas presentes son basifijas y se insertan por medio de 1–2 células cilíndricas o por todas las células de su base; aunque también aparecen protoscamas subpeltadas.

El estípite, el raquis y las costas son casi glabros. Las protoscamas de estos ejes son bastante uniformes en su morfología. Son pluricelulares, caducas, concolores, castañas, lineares o angostamente lanceoladas (Fig. 1I), lanceoladas típicas a lanceoladas anchas (Fig. 1H), elípticas grandes o pequeñas (Fig. 1C, U) u, ocasionalmente, irregulares (Fig. 1B).

En las cóstulas, costúlulas, venas secundarias y láminas se encuentran tricomas y protoscamas fimbriadas con cuerpo paucicelular o pluricelular prolongado en procesos 1–3-seriados cortos o largos. Los tricomas simples 1–2-seriados predominan en *Marattia alata* (Fig. 1J), mientras que los tricomas ramificados con 1–2 brazos generalmente 1-seriados, son característicos de *M. excavata* y *M. interposita* (Fig. 1G, L). Las protoscamas con cuerpos paucicelulares, escasamente ramificadas aparecen en *M. alata* (Fig. 1K), y las muy diversamente ramificadas con el cuerpo prolongado en procesos 1–pluriseriados largos en *M. excavata* (Fig. 1F), *M. interposita* (Fig. 1D), *M. laxa* (Fig. 1S) y *M. weinmanniifolia* (Fig. 1T, V). El indumento de estas especies se completa con protoscamas pluricelulares, lanceoladas angostas (Fig. 1A) o con cuerpo irregular prolongado en procesos irregulares o piliformes (Fig. 1E, Q).

En *Marattia laevis* se encuentran protoscamas 2–3-seriadas con el cuerpo ligeramente curvo y sin ramificar o poco ramificado, que aquí se han designado como acintadas (Fig. 1W, X). Esta especie presenta además acúleos o aguijones (Fig. 1P) que son tricomas pluricelulares epidérmicos, de forma cónica y aguda, no endurecidos ni esclerosados, ubicados espaciadamente en la cara adaxial, sobre costúlulas y venas secundarias (Font Quer, 1982). En *M. cicutifolia* se presentan protoscamas pluricelulares, irregulares, muy características, con cuerpos prolongados en ramificaciones cortas formadas por unas pocas células alargadas (Fig. 1M–O, R).

EPIDERMIS (FIGS. 2, 3)

En las especies neotropicales de *Marattia* las paredes de las células epidérmicas son relativamente delgadas y nunca hay engrosamientos localizados, los que sí están presentes en dos especies paleotropicales, *M. obesa* Christ y *M. silvatica* Blume (Rolleri et al., 2001a). En vista superficial, las epidermis adultas presentan contornos sinuosos, distantes, curvos o angulares de amplitud y profundidad variable y las células tienen aspecto de piezas de rompecabezas.

El ratio celular (relación largo/ancho de las células) es diferente en epifilos e hipofilos. Los epifilos presentan células isodiamétricas (ratio celular 1:1) sólo en *Marattia alata* (Fig. 2E), o células más largas que anchas (ratio celular 1.5–3:1) en el resto de las especies. En los hipofilos se encuentran ratios mayores: desde 2–3:1 (Figs. 2D, F, 3F), hasta 3–6:1 (Figs. 2B, H, 3B, D).

La combinación de los modelos epidérmicos y de los ratios celulares en epifilos e hipofilos es la siguiente:

- Epifilos con ondas angulares de amplitud más bien uniforme y escasa profundidad, y ratio celular 1.5–2:1; hipofilos con ondas curvas irregulares profundas, y ratio celular 3–6:1, en *Marattia cicutifolia* (Fig. 2A, B).
- Epifilos con ondas curvas y subangulares e irregulares, ratio celular 2:1; hipofilos con ondas cuadrangulares o curvas, profundas y ratio celular 2–3:1, en *Marattia excavata* (Fig. 2C, D).
- Epifilos con ondas curvas uniformes y poco profundas, y células isodiamétricas (ratio celular 1:1); hipofilos con ondas curvas irregulares, profundas a muy profundas, y ratio celular 2–3:1, en *Marattia alata* (Fig. 2E, F).
- Epifilos con ondas curvas no uniformes, profundas y someras, y ratio celular 1.5–3:1; hipofilos similares pero con ondas curvas de menor profundidad, y ratio celular 3–6:1, en *Marattia laxa* (Fig. 2G, H).
- Epifilos con ondas curvas profundas casi uniformes, y ratio celular 2:1; hipofilos con ondas curvas muy irregulares, profundas, y ratio celular 3–6:1, en *Marattia weinmanniifolia* (Fig. 3A, B).
- Epifilos con ondas curvas irregulares y en general profundas, ratio celular 3:1; hipofilos con ondas regulares y someras, ratio celular 3–4:1, en *Marattia interposita* (Fig. 3C, D).
- Epifilos e hipofilos muy similares entre si, con ondas curvas irregulares y profundas, y ratio celular 2–3:1, en ambos, en *Marattia laevis* (Fig. 3E, F).

ESTOMAS (FIG. 1)

El estoma maratiáceo, difundido en toda la familia, tiene contorno elíptico y es 2–3 veces más largo que ancho; las células oclusivas están sobrelevadas por encima de las epidérmicas adyacentes y tienen las paredes externas engrosadas. La membrana cuticular gruesa y pectinada de la pared externa de las oclusivas está reducida o desaparece en los polos, formando dos

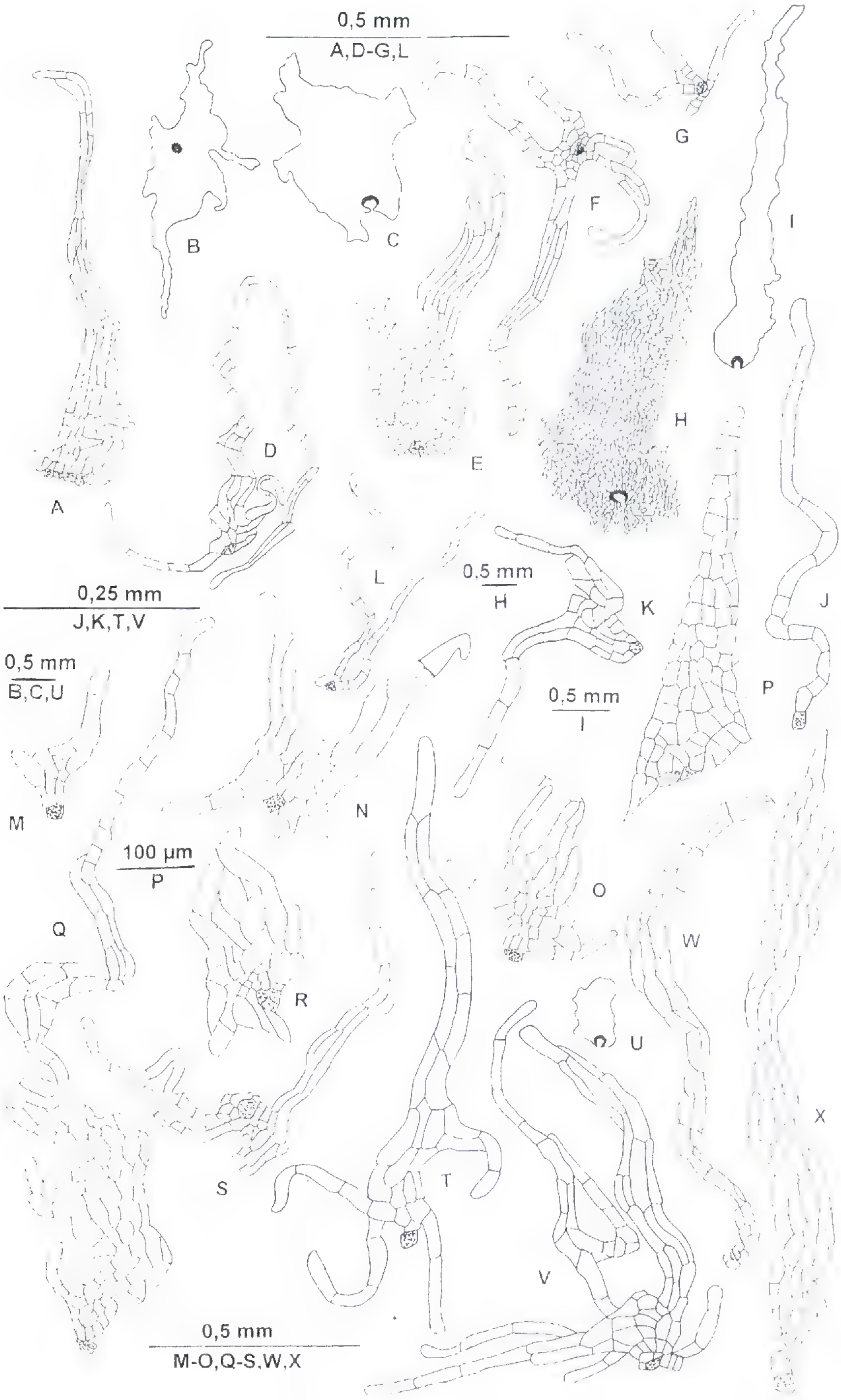

Figura 1. A–X. Indumento de raquis, costas, cóstulas, venas secundarias y superficie de la lámina en especies de *Marattia*. A–D. *M. interposita*. —A–C. Protoscamas pluricelulares. —D. Protoscama paucicelular. E–G. *M. excavata*. —E. Protoscama pluricelular. —F. Protoscama paucicelular. —G. Tricoma. H–K. *M. alata*. —H, I. Protoscamas pluricelulares. —J. Tricoma. —K. Protoscama paucicelular. —L. *M. excavata*: tricoma. —M–O. *M. cicutifolia*: protoscamas pluricelulares. —P. *M. laevis*: aguijón. —Q. *M. laxa*: protoscama paucicelular. —R. *M. cicutifolia*: protoscama pluricelular. —S. *M. laxa*: protoscama paucicelular. T–V. *M. weinmanniifolia*. —T, V. Protoscamas laciniadas. —U. Protoscama pluricelular. —W. X. *M. laevis*: protoscamas acintadas. En negro: zona de inserción de protoscamas; sombreado: célula basal.

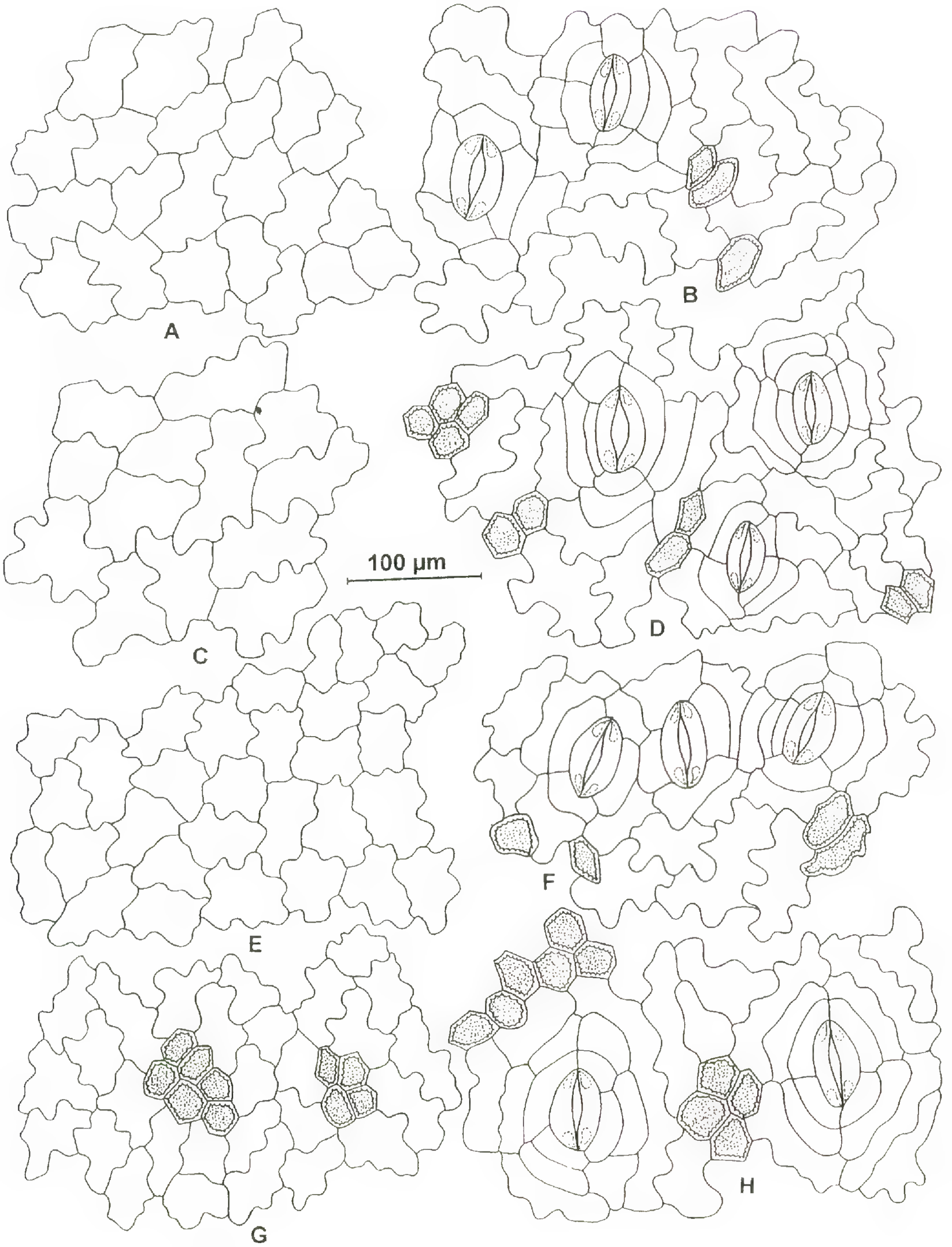

Figura 2. A–H. Modelos epidérmicos, estomas e idioblastos en especies de *Marattia*. —A–B. *M. cicutifolia*. —C–D. *M. excavata*. —E–F. *M. alata*. —G–H. *M. laxa*. A, C, E, G: superficie adaxial. B, D, F, H: superficie abaxial.

áreas deprimidas localizadas, con paredes primarias (Rolleri & Lavalle, 1997). El estoma está rodeado de 1–2 ciclos de anexas que determinan estomas adultos ciclocíticos con un ciclo interno bien delimitado, y un segundo ciclo en general incompleto o con más desarrollo de las anexas laterales que de las polares.

En *Marattia* las células oclusivas y las células anexas, en vista superficial y observadas con microscopio electrónico de barrido (MEB), pueden presentar filigranas, estrías o pliegues en la cutícula, tanto en especies neotropicales como paleotropicales (Rolleri & Lavalle, 1997).

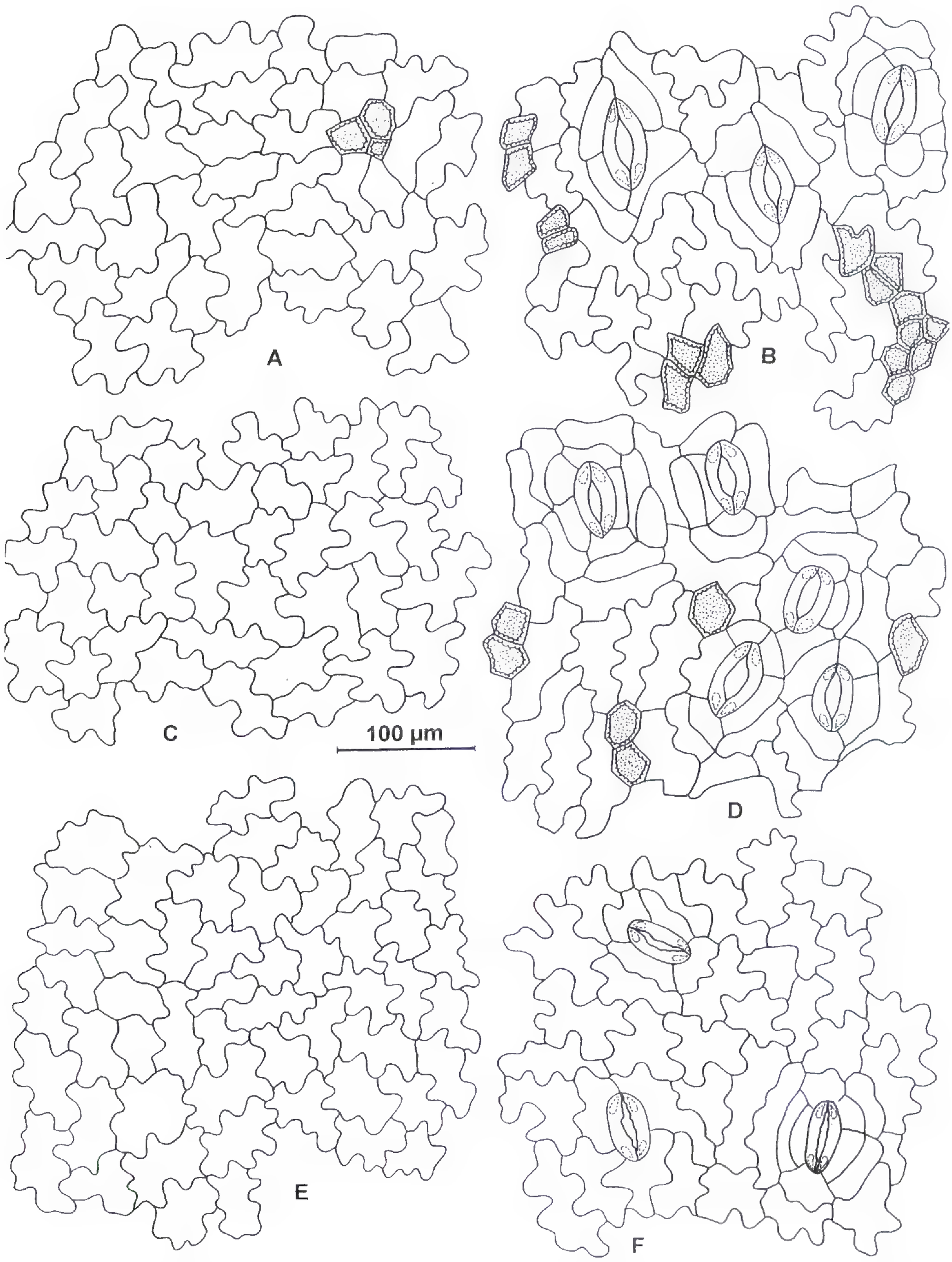

Figura 3. A–F. Modelos epidérmicos, estomas e idioblastos en especies de *Marattia*. —A–B. *M. weinmanniifolia*. —C–D. *M. interposita*. —E–F. *M. laevis*. A, C, E: superficie adaxial. B, D, F: superficie abaxial.

En las especies neotropicales de *Marattia*, en vista superficial, las áreas sobrelevadas, las piezas polares y el borde epidérmico de las oclusivas tienen una marcada concentración de pectinas entre la pared celulósica y la cutícula mientras que las áreas deprimidas son exclusivamente celulósicas.

El borde epidérmico de las oclusivas puede estar uniformemente engrosado de polo a polo del estoma (Fig. 4E, F, K); estar o no engrosado (Fig. 4B, C, I); o carecer de engrosamientos (Fig. 4A, J). En *Marattia cicutifolia* (Fig. 4C) el engrosamiento epidérmico puede limitarse solamente a la parte curva de la pared

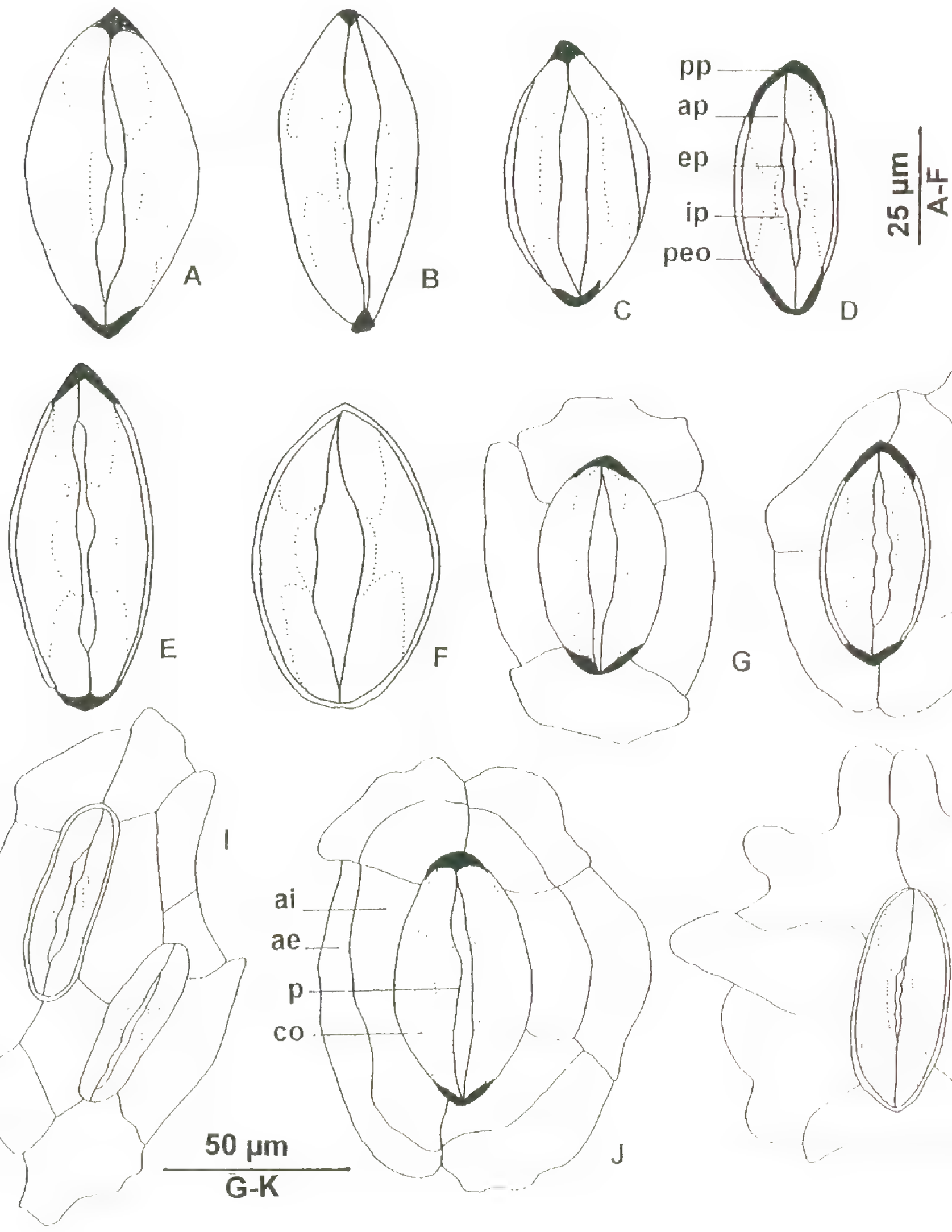

Figura 4. A–K. Caracteres de las células oclusivas y tipos de estomas adultos en especies de *Marattia*. —A. *M. laxa*. —B–C. *M. cicutifolia*. —D. *M. alata*. —E. *M. excavata*. —F. *M. weinmanniifolia*. —G. *M. cicutifolia*: estoma tetracítico. —H. *M. interposita*: estoma estaurocítico. —I. *M. alata*: estomas actinocíticos contiguos. —J. *M. laxa*: estoma ciclocítico. —K. *M. laevis*: estoma anisocítico. Las abreviaturas son: ae, anexas del ciclo externo; ai, anexas del ciclo interno; ap, área polar deprimida; co, célula oclusiva; ep, reborde externo del poro; ip, reborde interno del poro; p, poro; peo, pared epidérmica de la oclusiva; pp, pieza polar.

epidérmica de las células oclusivas, adelgazándose luego en el tramo cercano a las piezas polares.

Las piezas polares son frecuentes y aparecen en forma de cuña triangular (Fig. 4A, C) y en forma de "T" (Fig. 4D, E, H). Faltan en *Marattia laevis* (Fig. 4K) y *M. weinmanniifolia* (Fig. 4F). Los poros son de tamaño normal, de aproximadamente 1/3 de la longitud total del estoma en *M. laevis* (Fig. 4K), mientras que en las especies restantes los poros son muy grandes con una apertura que se extiende de polo a polo, haciendo contacto o no con una pieza polar (Fig. 4A, B, F, G). En *M. excavata* (Fig. 4E) el poro elíptico contacta externamente en dos puntos ("tri-aperturado"). En *M. alata* (Fig. 4D), *M. interposita* (Fig. 4H) y *M. laevis* (Fig. 4K) los bordes del poro son crenados u ondulados siendo lisos en las restantes especies.

Marattia, como otras Marattiaceae, presenta una cierta variedad de estomas ciclocíticos: actinocíticos (Figs. 3B, D, 4I), tetracíticos (Figs. 2B, F, 3D, 4G), estaurocíticos (Figs. 2H, 3B, D, 4H), anisocíticos característicos de *M. laevis* (Figs. 3F, 4K) y esporádicamente ciclocíticos típicos con dos ciclos completos de anexas (Figs. 2H, 4J).

El patrón cíclico de las anexas puede mostrar ciertas anomalías, como los estomas contiguos, relativamente frecuentes en *Marattia alata* (Fig. 4I),

Tabla 1. Tabla de dimensiones y densidades estómaticas en las especies neotropicales (n) y paleotropicales (p) de *Marattia*. Para cada especie se midieron 50 estomas; se estimaron las densidades de 50 hojas.

Especies	Largo × ancho en µm*	Densidad en número de estomas/ 0.25 mm² de superficie*	Densidad en número de estomas/mm² de superficie*
M. alata n	(42) 53 (67) × (34) 23 (17)	(8) 17 (30)	(32) 68 (120)
M. cicutifolia n	(62) 50 (39) × (44) 25 (17)	(2) 6 (15)	(8) 24 (60)
M. excavata n	(64) 59 (55) × (31) 28 (23)	(4) 9 (20)	(16) 36 (80)
M. fraxinea p	(36) 42 (48) × (22) 25 (30)	(7) 12 (17)	(28) 48 (68)
M. interposita n	(67) 55 (47) × (34) 28 (22)	(5) 13 (25)	(20) 52 (100)
M. laevis n	(64) 53 (33) × (28) 23 (19)	(4) 14 (21)	(32) 56 (92)
M. laxa n	(69) 58 (44) × (39) 31 (25)	(7) 11 (19)	(28) 44 (76)
M. obesa p	(37) 44 (47) × (23) 27 (31)	(5) 8 (12)	(20) 32 (48)
M. salicina p	(39) 43 (48) × (27) 29 (31)	(9) 14 (17)	(36) 56 (68)
M. smithii p	(36) 41 (45) × (22) 27 (30)	(13) 15 (21)	(52) 60 (84)
M. weinmanniifolia n	(72) 59 (48) × (41) 30 (17)	(7) 15 (23)	(28) 60 (92)
M. werneri p	(60) 48 (37) × (33) 22 (16)	(11) 14 (18)	(44) 56 (72)

* (mínimo) medio (máximo).

M. interposita y *M. laevis*; también se han observado con menor frecuencia estomas adyacentes.

Las dimensiones de los estomas varían ligeramente de una especie a otra y tienen aproximadamente 50–59 µm de largo por 23–31 µm de ancho. La densidad estomática promedio va desde 24 estomas/mm² en *Marattia cicutifolia* hasta 68 estomas/mm² en *M. alata*. El rango de variación es similar en las especies neotropicales y en la mayoría de las paleotropicales del género (Tabla 1).

IDIOBLASTOS (FIGS. 2, 3)

Tres géneros del orden Marattiales: *Angiopteris*, *Archangiopteris* y *Marattia* presentan sílice en forma de ópalo A en idioblastos epidérmicos (Rolleri et al., 2001b). En *M. douglasii* C. Presl (Baker), *M. fraxinea* Sm., *M. obesa*, *M. salicina* Sm., *M. smithii* Kuhn, *M. silvatica* y *M. werneri* Ros. (especies paleotropicales) predominan los idioblastos aislados o en grupos pequeños (Rolleri et al., 2001a).

Todas las especies neotropicales de *Marattia* con excepción de *M. laevis* (Fig. 3F) presentan idioblastos de ópalo en la epidermis. Son abaxiales y adaxiales en *M. laxa* (Fig. 2G, H) y *M. weinmanniifolia* (Fig. 3A, B) y exclusivamente abaxiales en las demás especies. Los idioblastos aparecen aislados o en grupos de 2 en *M. alata* (Fig. 2F), *M. cicutifolia* (Fig. 2B) y *M. interposita* (Fig. 3D); en grupos de 2–3(5) idioblastos en *M. excavata* (Fig. 2D); y formando además masas de hasta 9–10 idioblastos en *M. laxa* (Fig. 2H) y *M. weinmanniifolia* (Fig. 3B).

En las especies neotropicales los idioblastos suelen ser más pequeños que los estomas, circulares o poligonales, mientras que son iguales o más grandes que un estoma en la mayoría de las especies paleotropicales, observandose contornos circulares en *Marattia obesa*, poligonales en *M. salicina* e irregulares en *M. silvatica* (Rolleri et al., 2001a).

La densidad de los idioblastos es alta en *Marattia alata*, *M. excavata* y *M. laxa*; media en *M. interposita* y *M. weinmanniifolia*; y baja en *M. cicutifolia* donde hay extensas áreas de epidermis sin idioblastos.

CLAVE DE LAS ESPECIES NEOTROPICALES DEL GÉNERO *MARATTIA*

1. Láminas 2-pinnadas; pínnulas insertas en la costa a distancias similares y constantes . . . . . (2)
1. Láminas 3–4-pinnadas; pínnulas insertas en la costa a distancias acrópetamente decrecientes . . . (3)
2(1). Pínnula terminal de mayor tamaño que las laterales; venas subhorizontales, bifurcadas dos veces predominantes, escasas geminadas; sinangios intramarginales con receptáculos planos, no sobrelevados; ópalos abundantes en epifilos e hipofilos, en grupos de 2–3(9); células del epifilo e hipofilo con ondas curvas irregulares, profundas y someras en epifilos, poco profundas en hipofilos; 11 estomas/mm²; tricomas presentes, protoscamas paucicelulares ramificadas con brazos largos . . . . . *Marattia laxa*
2. Pínnula terminal igual que las laterales; venas oblicuas, geminadas predominantes, escasas bifurcadas; sinangios supramediales con receptáculos hiperdesarrollados cortos; ópalos escasos, sólo en hipofilo, aislados o en grupos de 2; células del epifilo con ondas angulosas uniformes y de escasa profundidad; células del hipofilo con ondas curvas irregulares y profundas; 24 estomas/mm²; tricomas ausentes, protoscamas pluricelulares irregulares con brazos cortos . . . *Marattia cicutifolia*
3(1). Venas exclusivamente simples o simples con escasas bifurcadas. Células del epifilo e hipofilo sólo con ondas curvas. Opalos presentes . . . . . (4)
3. Venas exclusivamente bifurcadas. Células del epifilo e hipofilo con ondas curvas o de otro tipo. Ópalos presentes o ausentes . . . . . (5)
4(3). Venas simples predominantes, escasas bifurcadas, nítidas; células del epifilo isodiamétricas (ratio celular = 1:1), con ondas curvas uniformes y poco profundas; ópalos, solo en hipofilo,

solitarios o en grupos de 2; estomas con piezas polares en forma de "T", bordes del poro crenados u ondulados; protoescamas paucicelulares escasamente ramificadas; sinangios intramarginales . . . . . *Marattia alata*

1. Venas exclusivamente simples, poco nítidas; células del epifilo más largas que anchas (ratio celular = 2:1), con ondas curvas uniformes y profundas; ópalos en epifilo e hipofilo, en grupos de 2–3(9); estomas sin piezas polares, bordes del poro lisos; protoescamas paucicelulares laciniadas; sinangios supramediales . . *Marattia weinmanniifolia*

5(3). Células del epifilo e hipofilo similares, sólo con ondas curvas profundas e irregulares; ópalos ausentes; estomas con poros de aproximadamente 1/3 de la longitud total del estoma y sin piezas polares; acúleos presentes sobre costúlulas; protoescamas acintadas, tricomas ausentes; sinangios supramediales con receptáculos hiperdesarrollados largos (pedicelos) . . . . . *Marattia laevis*

5. Células del epifilo e hipofilo distintas, con ondas curvas o de otro tipo; ópalos presentes, sólo en hipofilo; estomas con poros que se extienden casi de polo a polo y con piezas polares en forma de "T"; acúleos ausentes; protoescamas fimbriadas no acintadas, tricomas ramificados; sinangios intramarginales con receptáculos normales, no hiperdesarrollados . . . . . (6)

6(5). Pínnulas con ápice representado por un segmento inciso casi indiviso; pinnúlulas oblanceoladas con ápices obtusos; células del epifilo grandes con ondas curvas, subangulares o cuadrangulares; ópalos en grupos de 2–3(5); 36 estomas/mm², con poro interno triaperturado . . . *Marattia excavata*

6. Pínnulas con ápice representado por una pinnúlula terminal entera; pinnúlulas lanceoladas algo falcadas con ápices agudos; células del epifilo pequeñas sólo con ondas curvas; ópalos solitarios o en grupos de 2; 52 estomas/mm², con poro interno crenado . . . . . *Marattia interposita*

Conclusiones

• El conjunto de caracteres formado por el modelo epidérmico de epifilo e hipofilo, el tipo de estoma asociado, la densidad estomática, la presencia, agrupamiento y localización de los idioblastos constituye un rasgo diagnóstico específico.

• Las epidermis presentan modelos sinuosos distantes, formados por células con paredes uniformemente delgadas en las especies neotropicales y con engrosamientos locales en las sinuosidades en especies paleotropicales. Los modelos poligonales están ausentes en las especies neotropicales y sólo se presentan en *Marattia salicina* (paleotropical).

• Las especies neotropicales de *Marattia* presentan estomas maratiáceos ciclocíticos con poros elípticos muy grandes, con excepción de *M. laevis*; engrosamientos variables de la pared epidérmica de las células oclusivas, ausentes en *M. laxa*; y piezas polares en forma de "T" o "de cuña triangular" no presentes en *M. laevis* y *M. weinmanniifolia*. Los tipos estomáticos más frecuentes son los actinocíticos, tetracíticos y estaurocíticos; el anisocítico es característico de *M. laevis*. Las dimensiones de los estomas son similares en especies neotropicales y paleotropicales mientras que las densidades estomáticas son variables.

• Los idioblastos contienen ópalo A y están presentes en todas las especies de *Marattia* con excepción de *M. laevis*. Son exclusivamente hipofílicos en la mayoría de las especies y también epifílicos en *M. laxa*, *M. weinmanniifolia* y *M. obesa* (paleotrópico). *Marattia* se distingue por los idioblastos solitarios o reunidos en grupos pequeños mientras que *Angiopteris* y *Archangiopteris* pueden desarrollar extensas áreas de idioblastos.

• El indumento es demasiado uniforme para considerarlo un rasgo diagnóstico específico, si bien hay especies que se destacan por su indumento: *Marattia cicutifolia*, por sus protoescamas con cuerpos desarrollados y poco ramificados, semejantes a las protoescamas de *Angiopteris* (Rolleri, 2002); y *M. laevis* por sus protoescamas acintadas y sus acúleos o aguijones hipofílicos sobre costúlulas y venas secundarias. El resto de las especies presenta tricomas, protoescamas paucicelulares o pluricelulares con cuerpo muy dividido.

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CARACTERES DIAGNÓSTICOS FOLIARES EN *ISOETES* (PTERIDOPHYTA, ISOETACEAE)[1,2]

Cristina H. Rolleri[3] *and Carmen Prada*[4]

Resumen

Se han estudiado caracteres foliares de 25 taxones europeos, norteafricanos, norteamericanos y sudamericanos, terrestres, anfibios y acuáticos, del género *Isoetes* L. (Pteridophyta, Isoetaceae). Los caracteres morfológicos foliares investigados tienen un valor diagnóstico variable. Son específicos y deberían incluirse en las descripciones taxonómicas las ornamentaciones cuticulares, los tipos de márgenes, los modelos y las relaciones longitud/anchura (relaciones l/a) celulares de las células epidérmicas, los tipos de protuberancias intercelulares pécticas (PIP), los modelos y las relaciones l/a celulares de las células epidérmicas del velo, el modelo epidérmico adaxial de los esporangios y la naturaleza de sus paredes celulares. Son caracteres de grupos de especies los contornos de las secciones foliares, los tipos de colénquima (en haces, lacunar o parcialmente laminar, los dos últimos descritos aquí por primera vez), los tipos de células de los diafragmas, la presencia y tipos de PIP, la extensión de la endodermis en las cámaras aeríferas, la lígula secretora persistente o esclerosada tempranamente. Son genéricos la presencia de lígula secretora y los tipos de endodermis. Al género se le ha atribuido escasez de caracteres morfológicos o variación de éstos con el ambiente, dos conceptos que aquí se discuten ante las nuevas evidencias.

Abstract

Foliar characters were studied in 25 European, North African, North American, and South American terrestrial, amphibious, and aquatic taxa of the genus *Isoetes* L. (Pteridophyta, Isoetaceae). The foliar characters investigated have a variable diagnostic value. The presence and types of cuticular ornamentations, types of margins, epidermal patterns and cell ratios of the epidermis, types of IPP (intercellular pectic protuberances) in the cells of the leaf diaphragms, epidermal patterns and cell ratios of velum, epidermal patterns, and nature of walls in the adaxial epidermis of sporangia may be good diagnostic specific characters that should be included in taxonomic descriptions. Transverse sections of the microphylls, the types and distribution of collenchyma (fascicular, lacunar, or partially laminar collenchyma, with the latter two types described here for the first time), the cell types of leaf diaphragms, the presence of IPP in these cells, the partial or complete endodermis in the air chambers, and the presence of a persistent mucilage-secreting or an early lignified ligule are features that characterize species groups in *Isoetes*. The mucilage-secreting ligula and the types of endodermis can be considered as generic traits in *Isoetes*. The genus was largely considered as lacking good diagnostic characters or having rather environmentally influenced traits, two concepts that are discussed here after presentation of the new evidence.

Key words: foliar diagnostic characters, epidermis, *Isoetes*, microphylls, labium, ligula, sporangial adaxial wall, velum.

Los trabajos sobre morfología interna son escasos en *Isoetes* L. (Pteridophyta, Isoetaceae) y su aplicación a la sistemática del género no es frecuente. Los estudiados con más detalle han sido los relacionados con el cormo, una estructura poco variable en las especies del género. Algunos trabajos trataron caracteres foliares en general, además del cormo y el sistema vascular, siempre en relación con una o unas pocas especies (Scott & Hill, 1900; Stokey, 1909; West & Takeda, 1915).

Pfeiffer (1922) recopiló los datos de las especies conocidas hasta entonces y utilizó, entre otros rasgos anatómicos externos e internos, los estomas y los tejidos mecánicos hipodérmicos, a los que llamó "peripheral bast" y consideró de sostén. Ese trabajo representó un cambio de concepto en la clasificación infragenérica. A partir de él se abandonó la clasificación basada en las características del medio que se había utilizado hasta ese momento (Braun, 1862; Motelay & Vendryès, 1882; Baker, 1880) y se pasó

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a una sistemática basada en rasgos esporales. La mayoría de los caracteres anatómicos nunca fueron evaluados ni utilizados en la sistemática, por considerarlos influidos por el medio.

Hall (1971) revisó las especies ganeanas de *Isoetes* y analizó caracteres nuevos como la presencia de cuñas y verrugas cuticulares, los tipos de células de los diafragmas foliares, las protuberancias intercelulares pécticas (PIP), que designó como "minute acicular spines", el número de canales intrastelares y la epidermis adaxial del esporangio, aunque dudó del valor taxonómico de algunos de ellos. Prada (1979a) retomó muchos de los caracteres foliares propuestos por Hall (1971) y los aplicó a especies ibéricas de *Isoetes*. Kott y Britton (1985) analizaron el color de la epidermis adaxial del esporangio en especies norteamericanas de *Isoetes* y lo consideraron un carácter taxonómico válido porque se correlacionaba con otros caracteres específicos, aunque esos autores no estudiaron el origen del color.

Hickey (1986) y Taylor y Hickey (1992) interpretaron la presencia de algunos caracteres morfológicos y anatómicos de los microfilos, tales como los filopodios, las hojas transformadas en escamas, los cordones colenquimáticos subepidérmicos y la pigmentación esporangial de ciertos grupos de taxones del género como el resultado de la convergencia debida a los cambios de hábitat ocurridos durante la historia evolutiva de *Isoetes* y utilizaron dichos caracteres para proponer una nueva división infragenérica. Troia et al. (1999) estudiaron la anatomía foliar comparada de dos especies, *I. durieui* Bory e *I. histrix* Bory, pero la única diferencia que destacaron fue el mayor tamaño de las células oclusivas de los estomas de la primera. Romeo et al. (2000) estudiaron la estructura de los canales intrastelares y pusieron en evidencia, mediante fluorescencia inducida por colorantes, la endodermis con bandas de Caspary presente en los mismos. Musselman y Roux (2002) ilustraron por primera vez, con microscopía electrónica de barrido, las ornamentaciones cuticulares con forma de verrugas, en *I. toximontana* L. J. Musselman & J. P. Roux.

Prada y Rolleri (2003) estudiaron comparativamente varios caracteres foliares en taxones ibéricos de *Isoetes* y analizaron rasgos como ornamentaciones cuticulares, modelos epidérmicos y estomas, secciones transversales foliares, distribución del colénquima subepidérmico, diafragmas foliares y presencia o ausencia de PIP. Rolleri y Prada (2004) estudiaron la endodermis de los canales intrastelares y describieron la endodermis de las cámaras aeríferas, y Prada y Rolleri (2005) dieron a conocer un estudio comparativo de las PIP y su valor diagnóstico en especies de *Isoetes*, en el que aplicaron por primera vez un conjunto amplio de caracteres morfológicos externos e internos en la diagnosis de *I. anatolica* Prada & Rolleri.

A la vista de los resultados obtenidos en el estudio de las especies ibéricas (Prada & Rolleri, 2003) y en la hipótesis de que los caracteres foliares son diagnósticos y el mejor conocimiento de su variación podría ayudar a la elaboración de una sistemática más natural que la basada principalmente en las esporas, se consideró importante extender el análisis de esos caracteres a un grupo más amplio de especies. Se eligieron especies europeas, norteafricanas, sudamericanas y norteamericanas, que crecen en diferentes ambientes y a distintas alturas sobre el nivel del mar. Los taxones estudiados fueron *Isoetes adspersa* A. Braun, *I. anatolica*, *I. andina* Spruce ex Hook., *I. boliviensis* U. Weber, *I. boryana* Durieu, *I. brochonii* Motelay, *I. durieui*, *I. echinospora* Durieu, *I. engelmannii* A. Braun, *I. histrix*, *I. howellii* Engelm., *I. killipii* C. V. Morton, *I. lacustris* L., *I. lechleri* Mett., *I. longissima* Bory, *I. malinverniana* Ces. & De Not., *I. melanopoda* J. Gay & Durieu, *I. novo-granadensis* H. P. Fuchs, *I. nuttallii* A. Braun ex Engelm., *I. orcuttii* A. A. Eaton, *I. piedmontana* (N. Pfeiffer) C. F. Reed, *I. setacea* Lam., *I. storkii* T. C. Palmer, *I. velata* A. Braun subsp. *velata* e *I. velata* A. Braun subsp. *asturicensis* (Laínz) Rivas Mart. & Prada.

Los caracteres fueron estudiados comparativamente en todas las especies y se aplicaron a tres casos diferentes para mostrar su utilidad: (1) en el caso de tres especies terrestres del oeste de Estados Unidos de América, que se han encontrado conviviendo en la misma localidad y ambiente, *Isoetes howellii*, *I. nuttallii* e *I. orcuttii*, las dos últimas, morfológicamente similares (Taylor et al., 1993); (2) en el grupo formado por *I. brochonii*, *I. echinospora* e *I. lacustris*, todas especies acuáticas, fenotípicamente muy similares, cuya determinación es difícil debido a sus relaciones de parentesco (Taylor & Hickey, 1992) y que a menudo conviven en los mismos lagos, y (3) en el grupo formado por *I. adspersa*, *I. boryana*, *I. longissima*, *I. velata* subsp. *velata* e *I. velata* subsp. *asturicensis*, que incluye especies morfológicamente similares, anfibias a casi terrestres o de aguas corrientes, con los mismos tipos de ornamentaciones esporales y con *status* taxonómico dudoso (Prelli, 2001).

Materiales y Métodos

En este trabajo se utilizó material de herbario restaurado, rehidratado o fresco, de recolecciones propias y de los herbarios COI, JACA, MA, MACB y SEV (Holmgren et al., 1990). Los pliegos que se citan en el Apéndice 1 contienen varios ejemplares, todos

los cuales han sido estudiados. Las colecciones citadas como *Prada s. n.* incluyen como mínimo 20 plantas, todas las cuales han sido estudiadas. Las observaciones se realizaron con microscopios óptico y electrónico de barrido. La restauración del material de herbario es conveniente especialmente para el estudio de las secciones foliares y se llevó a cabo de acuerdo con Prada y Rolleri (2003).

Para estudios de epidermis en superficie los microfilos se diafanizaron con hidróxido de sodio 2% acuoso, se blanquearon con hipoclorito de sodio comercial diluido 1:1, se lavaron repetidamente con agua corriente, se colorearon y se montaron en glicerina. Para los estudios de epidermis en superficie con ejemplares de herbario el material puede diafanizarse sin necesidad de restauración previa. Para estudios de secciones foliares se efectuaron cortes de los microfilos a mano alzada, se colorearon y se observaron montados en glicerina. La preparación de las muestras procedentes de material de herbario, para observar epidermis adaxial del esporangio y del velo, lígula y labio, se llevó a cabo sumergiendo los esporofilos durante 5–10 minutos en hidróxido de sodio 6% acuoso para ablandarlos y desprender los tejidos con más facilidad mediante pinzas de punta fina.

Todas las muestras se observaron con microscopio óptico, coloreadas con TBO 1% acuoso (Gurr, 1966). El TBO es un colorante metacromático, catiónico, transitorio, que colorea los tejidos con carga negativa. Es conveniente un breve lavado con agua acidificada con unas gotas de ácido acético para obtener una diferenciación más rápida. Las paredes primarias de las epidermis en general, las paredes pectinadas (PIP, colénquima) y las células secretoras del margen de la lígula se colorean de rosa a púrpura, mientras que las paredes celulósicas o el almidón no se colorean; además, en el caso de las células secretoras, sus núcleos se tiñen intensamente de color verde por la presencia de ácidos nucleicos y polifosfatos. En presencia de taninos o fenoles, predomina la reacción del colorante con los polifenoles, dando un color verde intenso. En las paredes secundarias lignificadas o suberificadas da color azul, azul-verdoso o azul brillante.

También se utilizó un microscopio de fluorescencia Zeiss con el fin de comprobar la fluorescencia intrínseca. La fluorescencia intrínseca se atribuye principalmente a la presencia de metabolitos secundarios y puede resultar útil en la determinación de ciertas sustancias, como la lignina, que emite autofluorescencia verdosa (Ruzin, 1999: 174). Aquí se utilizó para detectar las endodermis y para determinar si la reacción de color verde de ciertas células de la epidermis de los esporangios era debida a la presencia de polifenoles como precursores de taninos o de lignina.

Las medidas de los estomas y las relaciones longitud/anchura (relaciones l/a) se tomaron en todos los ejemplares estudiados. Los valores de las relaciones l/a celulares representan la media de las medidas tomadas en todas las células presentes en 10 campos elegidos al azar, tanto en epifilos como en hipofilos, observados con 200 aumentos (ca. 0.25 mm^2). Las dimensiones de los estomas representan la media de 25 medidas por cada microfilo, efectuadas en todos los ejemplares revisados. Las ilustraciones de los diversos aspectos de la morfología interna se realizaron con un microscopio Olympus CX 41 provisto de cámara clara.

Para los estudios con microscopio electrónico de barrido los fragmentos de microfilos procedentes de material fresco o restaurado se sometieron a fijación en punto crítico, se montaron sobre los soportes en plata coloidal y durofix, se metalizaron con oro bajo vacío y se fotografiaron con un microscopio JEOL JSM–T330A a 15 kV. En algunas especies las PIP y la endodermis de las cámaras aeríferas se observaron también en material de herbario simplemente rehidratado para ablandar los fragmentos de microfilos y poder abrirlos longitudinalmente para exponer al exterior dichas estructuras.

La terminología referente a la epidermis, PIP, secciones foliares y endodermis se ha utilizado siguiendo los criterios de Prada y Rolleri (2003) y Rolleri y Prada (2004), pero en el caso de las ornamentaciones cuticulares se ha preferido aquí no distinguir entre estrías y pliegues, y calificar como estrías a todas las ornamentaciones positivas, longitudinalmente alargadas, de sección curva o aguda, de espesor y continuidad variables.

Resultados

Los microfilos de las especies estudiadas de *Isoetes* son ensiformes, nacen helicoidalmente sobre el cormo y tienen sección transversal variable. Los microfilos externos son los más maduros, mientras que los más internos son juveniles. La base está ensanchada y presenta una expansión membranosa o ala que se prolonga acrópetamente con mayor o menor extensión. Están recubiertos por una epidermis cutinizada e internamente presentan un mesofilo provisto de cuatro cámaras aeríferas interrumpidas por diafragmas transversales formados por células globosas, lobuladas o estrelladas, diferentes de las del resto del mesofilo. Con frecuencia se presenta tejido mecánico de tipo colenquimático, diversamente distribuido y de posición hipodérmica.

En la base de la cara adaxial de los microfilos se forma la lígula, una pequeña lengüeta foliosa adpresa con el microfilo. Está separada del esporangio por un

labio más o menos desarrollado. El esporangio está inmerso en la fóvea basal del microfilo, cubierto o no por un velo. Los microfilos externos presentan generalmente macrosporangios y los más internos, generalmente microsporangios. La presencia y madurez de los esporangios depende del grado de desarrollo de las plantas y del momento de la estación de crecimiento en que se encuentran.

EPIDERMIS DE LOS MICROFILOS EN SUPERFICIE

Ornamentaciones y cuñas cuticulares. Las células epidérmicas de las especies estudiadas de *Isoetes* están cutinizadas. En vista superficial, tienen contorno poligonal. La pared externa es lisa u ornamentada y las ornamentaciones que se han observado consisten de estrías o verrugas. Las estrías son arrugas paralelas, finas o gruesas, que discurren longitudinalmente pasando o no por encima de las paredes de contacto transversales de las células epidérmicas. Las verrugas son protuberancias discretas, de forma variable, esféricas a elipsoidales, que sobresalen notablemente y se encuentran por lo general sobre estrías cuticulares. Todos los rasgos de la cutícula se observan bien tanto en superficie como en cortes transversales y mediante microscopio óptico o electrónico de barrido. Se colorean y destacan claramente con TBO. Son visibles por igual en microfilos centrales o periféricos del cormo y se forman tempranamente en los microfilos juveniles de las plántulas.

Las cutículas son lisas o casi lisas en *Isoetes anatolica* (Fig. 1C), *I. boliviensis*, *I. echinospora* (Fig. 1B), *I. engelmannii*, *I. howellii*, *I. lechleri*, *I. malinverniana*, *I. melanopoda*, *I. orcuttii* e *I. piedmontana* (Fig. 1A). Estrías irregulares, frecuentemente discontinuas, se han observado en *I. andina* (Fig. 2A), *I. brochonii* (Fig. 1E), *I. killipii* (Fig. 1F), *I. nuttallii* e *I. storkii* (Fig. 1D). Estrías regulares, continuas, que ocupan el centro o la totalidad de las paredes externas de las células epidérmicas, son características de *I. adspersa* (Fig. 3B), *I. boryana* (Fig. 2C, D), *I. longissima*, *I. novo-granadensis* (Fig. 3A), *I. setacea* (Fig. 3E), *I. velata* subsp. *velata* (Fig. 2E, F) e *I. velata* subsp. *asturicensis*. Las estrías más regulares, dispuestas siempre en un grupo central en la pared externa, son típicas de *I. boryana*, *I. longissima* e *I. velata* y las más finas, dispuestas homogéneamente en toda la pared externa, las de *I. setacea*. En *I. adspersa* e *I. novo-granadensis* las estrías están muy próximas, son gruesas y ocupan también toda la pared externa. Estrías cortas y prominentes, continuas o interrumpidas, se han observado en *I. durieui* (Fig. 3F), *I. histrix* e *I. lacustris* (Fig. 3C, D); en las dos primeras además, con abundantes verrugas cuticulares que están ausentes en *I. lacustris*.

Las paredes de contacto longitudinales de las células epidérmicas pueden desarrollar cuñas cuticulares externamente prominentes y muy engrosadas (Fig. 2B), que aparecen como un gran pliegue longitudinalmente continuo en *Isoetes adspersa*, *I. anatolica*, *I. andina*, *I. boryana*, *I. durieui*, *I. histrix*, *I. howellii*, *I. killipii*, *I. lacustris*, *I. novo-granadensis*, *I. nuttallii*, *I. piedmontana*, *I. setacea* e *I. storkii*. En el resto de las especies no hay cuñas externas sobresalientes.

Modelos epidérmicos. En vista superficial, las células epidérmicas tienen paredes rectas y se disponen con un modelo poligonal en todos los taxones estudiados. La epidermis de los microfilos puede presentar variaciones en la relación l/a celular y en el tamaño de las células.

Las células epidérmicas pueden ser desde casi isodiamétricas, con relación l/a celular 1:1, hasta rectangulares y angostas, con relación l/a 14:1 (Tabla 1). Las relaciones l/a celulares más bajas, entre 1:1 y 1.5:1 se encontraron en *Isoetes boryana* (Fig. 4F); relaciones l/a celulares entre 2:1 y 4:1 se encontraron en *I. adspersa* (Fig. 4K), *I. anatolica*, *I. boliviensis* (Fig. 4L), *I. echinospora*, *I. lechleri* (Fig. 4B), *I. longissima*, *I. malinverniana* (Fig. 4H), *I. novo-granadensis* (Fig. 4G), *I. storkii* (Fig. 4A), *I. velata* subsp. *velata*, *I. velata* subsp. *asturicensis*; entre 5:1 y 7:1 en *I. andina* (Fig. 4C), *I. killipii* (Fig. 4D, E), *I. nuttallii*, *I. orcuttii* e *I. setacea*; y finalmente, las relaciones l/a celulares mayores, entre 8:1 y 14:1 se observaron en *I. brochonii*, *I. durieui*, *I. engelmannii* (Fig. 4I), *I. histrix*, *I. howellii*, *I. lacustris*, *I. melanopoda* (Fig. 4M, N), e *I. piedmontana* (Fig. 4J).

En la epidermis pueden aparecer al azar células o grupos celulares con paredes más engrosadas. Estos grupos están formados por unas 3–8 células, con paredes más gruesas que las de células adyacentes y suelen contener taninos. Se intercalan entre las células epidérmicas comunes y se aprecian con escaso aumento o con microscopio estereoscópico. Se observaron como rasgo muy característico y son abundantes en *Isoetes brochonii*, *I. lacustris* e *I. lechleri* (Fig. 4B), pero aparecen, más escasos, en otras especies, como *I. adspersa*, *I. longissima* e *I. velata*.

Los microfilos de *Isoetes histrix* se destacan por los márgenes papilosos (Prada & Rolleri, 2003). Las células de estos márgenes se observan en superficie como pequeños dentículos romos. *Isoetes durieui*, una especie similar a *I. histrix* en la ornamentación cuticular, tiene márgenes formados por células más

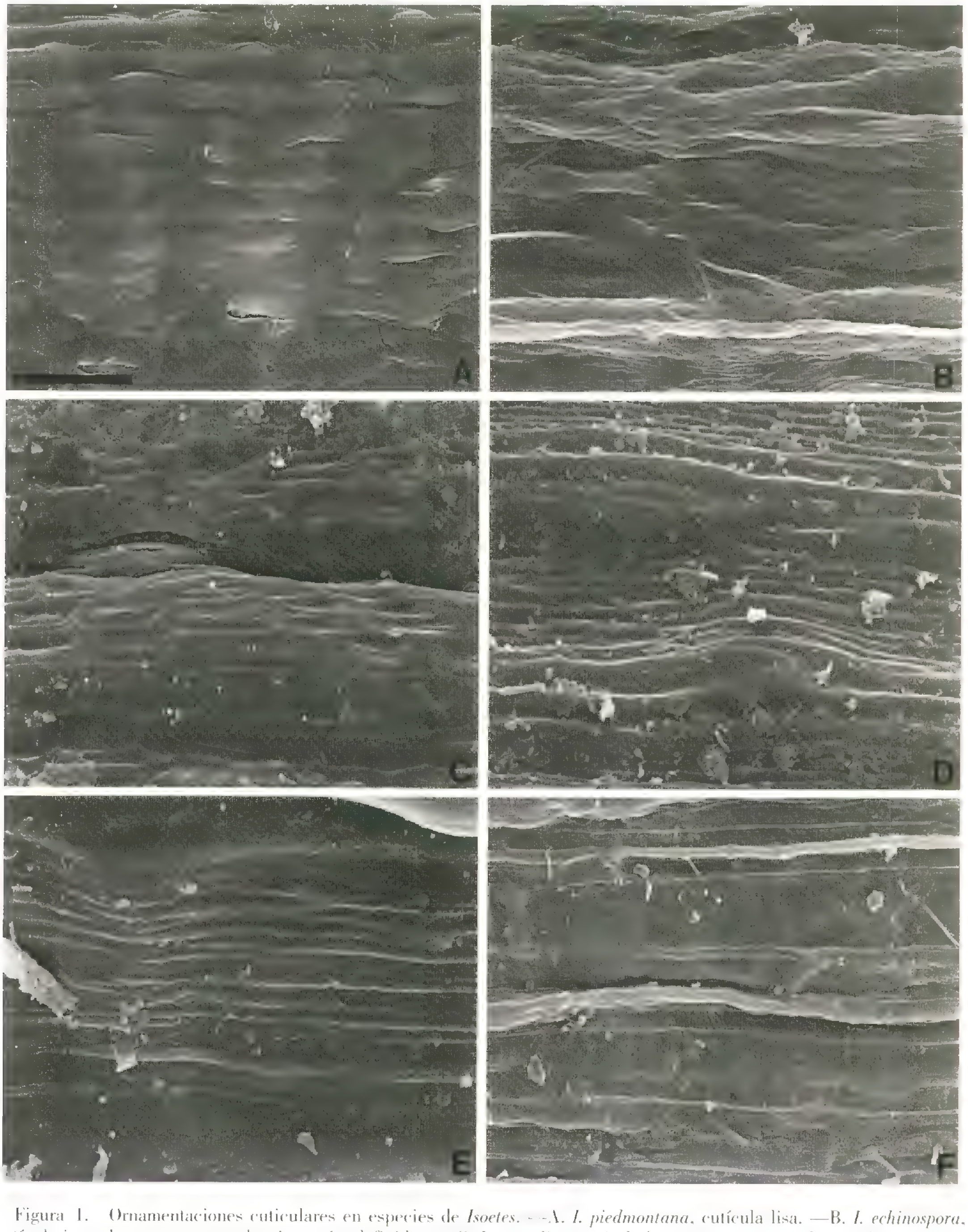

Figura 1. Ornamentaciones cuticulares en especies de *Isoetes*. —A. *I. piedmontana*, cutícula lisa. —B. *I. echinospora*, cutícula irregularmente engrosada, sin estrías definidas. —C. *I. anatolica*, cutícula lisa con cuñas cuticulares sobresalientes. —D. *I. storkii*, cuñas cuticulares sobresalientes y cutícula con estrías irregulares. —E. *I. brochonii*, cutícula con estrias irregulares. —F. *I. killipii*, cuñas cuticulares sobresalientes y cutícula con estrías irregulares. Escala: 50 µm en A, C, F; 20 µm en B; 10 µm en D, E.

engrosadas pero no son papilosos ni sobresalientes y el carácter se aprecia mejor en corte transversal.

Estomas. Los microfilos de las especies estudiadas son anfistomáticos o carecen de estomas. La ausencia de estomas se registró en algunas especies acuáticas o que viven permanentemente sumergidas, como *Isoetes brochonii*, *I. lacustris* e *I. lechleri*. Tampoco se presentan estomas o son muy escasos en algunas especies de los páramos, como *I. andina*, *I. boliviensis*,

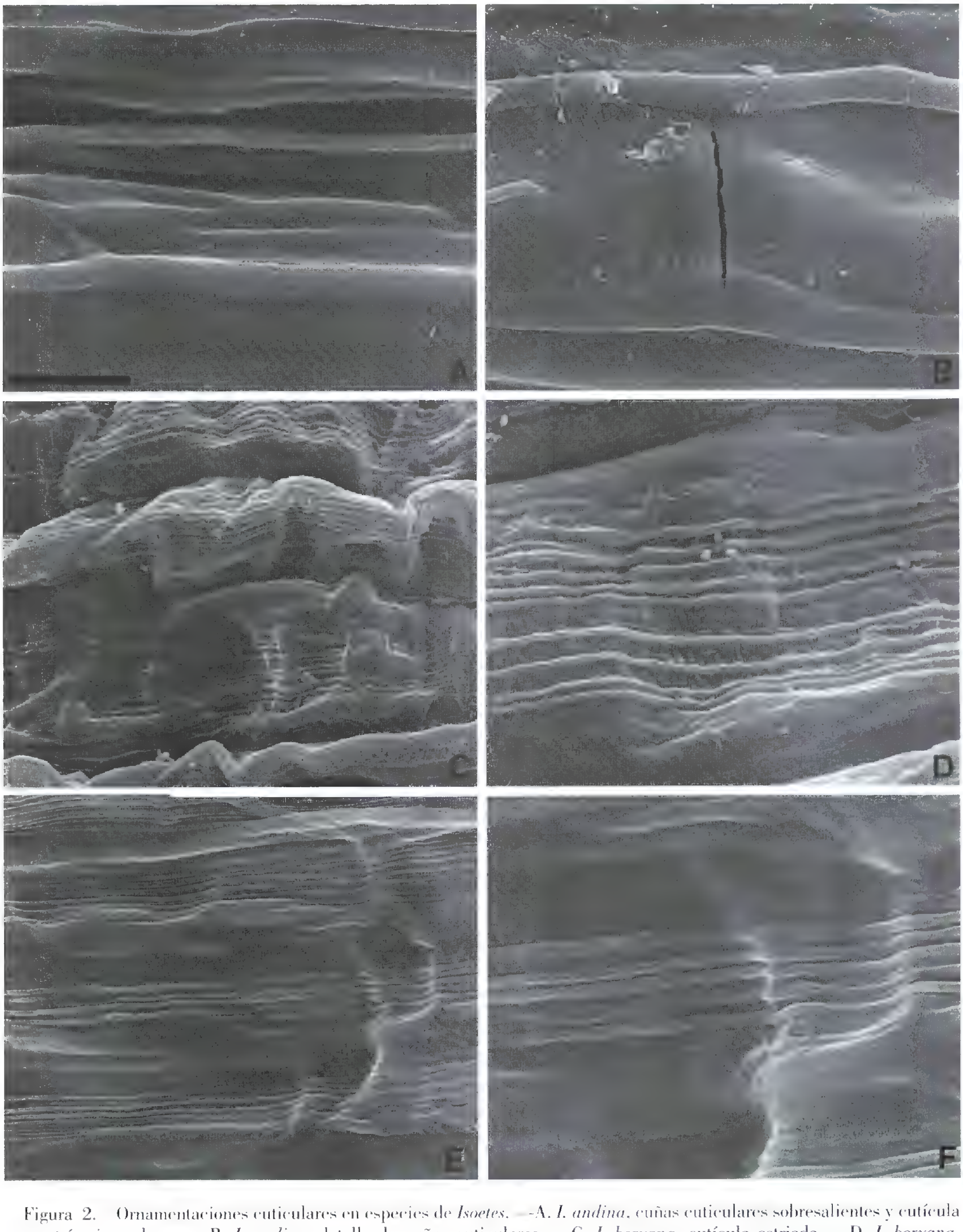

Figura 2. Ornamentaciones cuticulares en especies de *Isoetes*. —A. *I. andina*, cuñas cuticulares sobresalientes y cutícula con estrías irregulares. —B. *I. andina*, detalle de cuñas cuticulares. —C. *I. boryana*, cutícula estriada. —D. *I. boryana*, detalle de estrías regulares y paralelas. —E. *I. velata*, cutícula regularmente estriada. —F. *I. velata*, detalle de estrías centradas en pared externa. Escala: 20 μm en A, B, C, E; 10 μm en D, F.

I. killipii, *I. novo-granadensis* e *I. storkii*. En *I. killipii* los estomas están restringidos al tercio apical de las hojas y tal vez esto se relacione con el grado de emergencia de las mismas.

En la mayoría de las especies los estomas se distribuyen en dos bandas adaxiales y dos abaxiales a lo largo de toda la hoja. El número de hileras de estomas en las bandas puede variar entre 4–5(7)

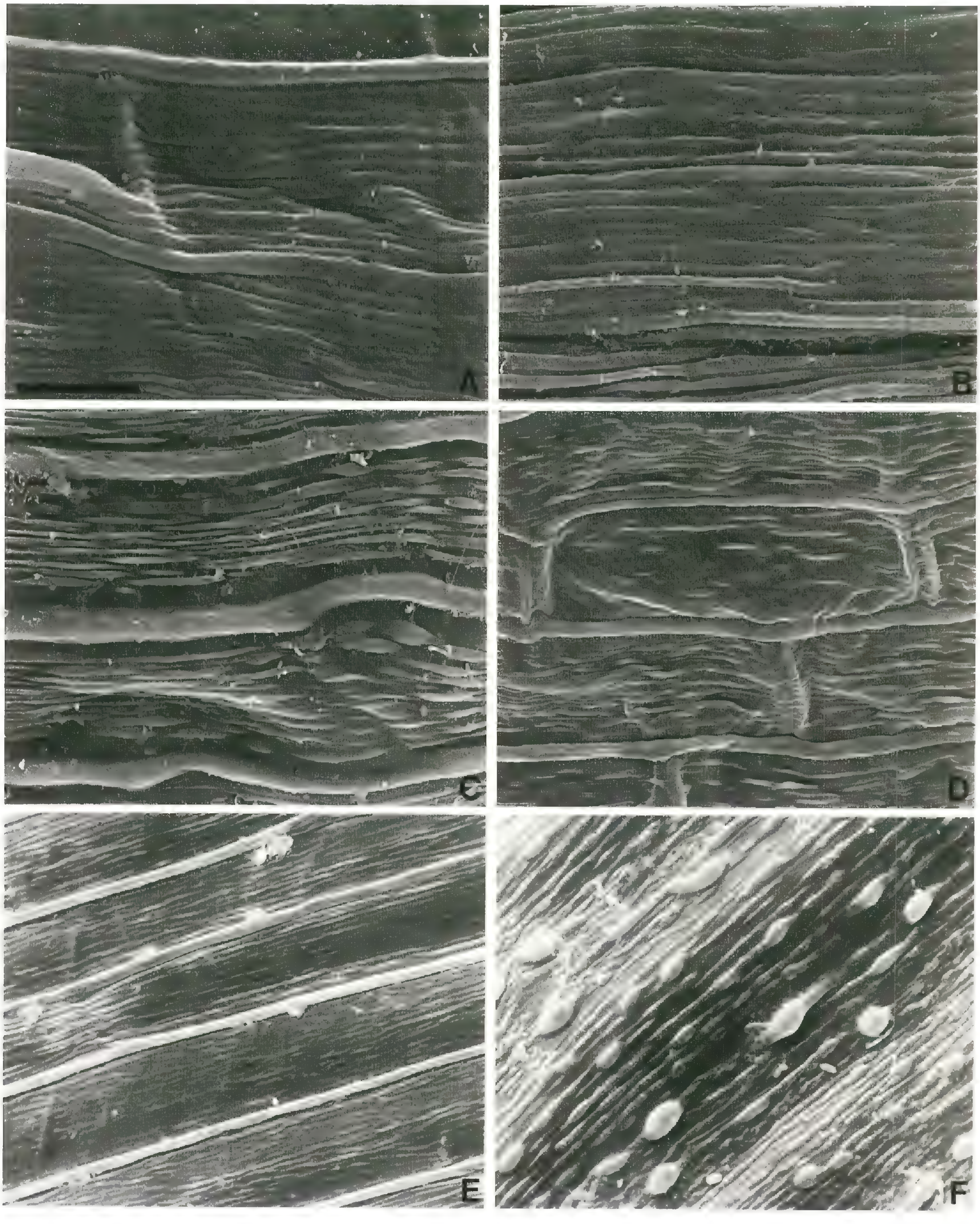

Figura 3. Ornamentaciones cuticulares en especies de *Isoetes*. —A. *I. novo-granadensis*, cuñas cuticulares sobresalientes y cutícula con estrías gruesas. —B. *I. adspersa*, cuñas cuticulares sobresalientes y cutícula con estrias gruesas. —C, D. *I. lacustris*, cuñas cuticulares sobresalientes y cutícula con estrías irregulares, gruesas y discontinuas. —E. *I. setacea*, cuñas cuticulares regulares prominentes y cutícula con estrías irregulares, muy finas y discontinuas. —F. *I. durieui*, cutícula con estrías gruesas, discontinuas y verrugas cuticulares. Escala: 10 µm en A, 50 µm en B; 20 µm en C, D; 15 µm en E, F.

hileras en las bandas adaxiales y 2–3(4) hileras en las abaxiales. Las células epidérmicas de las bandas estomáticas suelen tener menor relación l/a celular que las del resto de la epidermis.

Los estomas adultos tienen contorno casi orbicular en *Isoetes anatolica* e *I. boryana*, o contorno elíptico angosto en las restantes especies, en las que son 2–4 veces más largos que anchos. Tienen 4–8 células

Tabla 1. Caracteres foliares en especies de *Isoetes*.

Especie	Rce (1)	Rcv (2)	Dimensiones estomas (3)	PIP (4)	Colénquima (5)	Ploidía (6)
I. adspersa	3–4:1	12–13:1	(53) 60 (70) × (22) 25 (28)	v	haces	sin datos
I. anatolica	3:1	sin velo	(50) 54 (58) × (32) 35 (38)	f, c	haces	sin datos
I. andina	5:1	3:1	sin estomas	v	haces + chc	sin datos
I. boliviensis	1:1	1:1	(45) 50 (60) × (12) 15 (30)	sin PIP	sin col.	2*x* (Hickey, 1984)
I. boryana	1.5:1	4:1	(47) 57 (63) × (33) 38 (42)	sin PIP	haces	4*x* (Prada, datos no publicados)
I. brochonii	10–14:1	1:1	sin estomas	v, f	sin col.	12*x* (Taylor & Hickey, 1992)
I. durieui	6–9:1	2:1	(65) 70 (80) × (27) 33 (49)	v, f	haces	10*x* (Giovannini et al., 2001)
I. echinospora	3–4:1	3:1	sin estomas	sin PIP	haces	2*x* (Kay, 1974; Prada, 1979b; Taylor & Luebke, 1988)
I. engelmannii	8–9:1	4:1	(58) 62 (65) × (28) 31 (33)	v	sin col.	2*x* (Mathews & Murdy, 1969; Winstead & Musselman, 1994; Bar & Musselman, 1997; Brunton & Britton, 1997)
I. histrix	7–8:1	3:1	(35) 54 (59) × (22) 24 (30)	sin PIP	haces	2*x* (Manton, 1950; Prada, 1983) (*)
I. howellii	9–10:1	4:1	(52) 66 (80) × (20) 24 (30)	v, f	haces	2*x* (Britton & Brunton, 1995)
I. killipii	4.5–5:1	2:1	(62) 73 (87) × (30) 38 (45)	v	sin col.	sin datos
I. lacustris	8–10:1	2:1	sin estomas	v, f	sin col.	10*x* (Rychlewski & Jankun, 1972; Kott & Britton, 1980)
I. lechleri	4:1	3:1	sin estomas	v	clac	4*x* (Jermy, 1990; Troia, 2001)
I. longissima	2–5:1	3–4:1	(50) 63 (70) × (12) 17 (22)	v	haces	4*x* (Prada, 1983)
I. malinverniana	4:1	sin velo	(50) 64 (72) × (22) 30 (37)	f, c	haces	4*x* (Schneller, 1982)
I. melanopoda	7–8:1	5–6:1	(43) 58 (67) × (22) 27 (33)	v	haces + clac	2*x* (Brunton et al., 1996; Taylor et al., 1993)
I. novo-granadensis	3:1	2:1	(57) 68 (80) × (27) 32 (40)	sin PIP	sin col.	12*x* (Hickey, 1984)
I. nuttalii	6–7:1	4:1	(50) 63 (82) × (13) 20 (25)	v	haces	2*x* (Taylor et al., 1993)
I. orcutii	6:1	3:1	(50) 56 (65) × (17) 19 (22)	sin PIP	clac	2*x*, 4*x* (Taylor et al., 1993)
I. piedmontana	8–9:1	1:1	(47) 57 (63) × (22) 26 (30)	v	haces + clac	2*x* (Matthews & Murdy, 1969; Brunton et al., 1996; Taylor et al., 1993)
I. setacea	5–7:1	sin velo	(70) 77 (89) × (34) 38 (42)	v, f	haces	2*x* (Fernández Casas, 1977)
I. storkii	3:1	2:1	sin estomas	v	chc	2*x* (Hickey, 1984)
I. velata asturicensis	2–3:1	4:1	(37) 45 (62) × (13) 20 (30)	v	haces	2*x* (Prada, 1979b)

Table 1. Continued.

Especie	Ree (1)	Rev (2)	Dimensiones estomas (3)	PIP (4)	Colénquima (5)	Ploidía (6)
I. velata velata	2–3:1	1:1	(40) 57 (65) × (15) 16 (20)	v	haces	2x (Prada, 1979b)

1. Relaciones l/a celulares de la epidermis.
2. Relaciones l/a celulares del velo.
3. Dimensiones de los estomas (largo por ancho, en µm). Valores de: (promedio de los mínimos de todas las muestras de cada especie) promedio (promedio de los máximos de todas las muestras de cada especie).
4. Tipos de protuberancias intercelulares pécticas (PIP): c, conexiones; f, filamentos; v, verrugas.
5. Colénquima: haces (haces discretos, disposición 3 + 1, 3 + 1 y haces más pequeños, menos de 3 haces, etc.); chc, capa hipodérmica continua; clac, capa hipodérmica lacunar; sin col., sin colénquima de ningún tipo.
6. Se incluye el nivel de plodía, cuando se conoce, con las referencias bibliográficas.

(*) Manton (1950) registra un $x = 10$ [$2n = 20$], aunque en *Isoetes* en general predomina un $x = 11$. En esta especie Prada (1983) registra $2n$ 22. Todos los datos sugieren un diploide.

periestomáticas vecinas indistinguibles morfológicamente de las epidérmicas adyacentes, que se ordenan con disposiciones aproximadamente bipolares, más o menos tetracíticas o hexacíticas, o bien se disponen formando una corona de células más cortas y angostas, de manera más bien actinocítica. Esta última disposición es típica de *I. adspersa*, *I. longissima*, *I. velata* subsp. *velata* e *I. velata* subsp. *asturicensis* y también aparece, aunque puede ser menos clara, en *I. killipii*, *I. malinverniana*, *I. melanopoda*, *I. novogranadensis* e *I. piedmontana*.

Los estomas se diferencian tempranamente y maduran con rapidez. En *Isoetes velata* subsp. *velata* se han encontrado estomas completamente desarrollados en microfilos de 1–2 cm y en plántulas de tamaño aún menor. La orientación bipolar o actinocítica de las células periestomáticas también es temprana. Las escasas iniciales estomáticas y los estomas inmaduros hallados en ejemplares de *I. velata* sugieren que la vía de desarrollo es perígena, algo observado previamente por Prada y Rolleri (2003).

Las dimensiones estomáticas son variables y van desde 45 × 20 µm en *Isoetes velata* subsp. *asturicensis* hasta 77 × 38 µm en *I. setacea* (Tabla 1).

Secciones transversales foliares. Los microfilos son crasos, más o menos triquetros, cuadrangulares o casi teretes. Se atenúan gradualmente con un ápice más fino e igualmente craso, que en ciertas especies puede estar tapizado por una epidermis cuyas células presentan paredes más engrosadas que las restantes epidérmicas del microfilo y que pueden estar coloreadas de castaño.

En la parte media de las hojas el contorno de las secciones es más o menos trapezoidal en *Isoetes adspersa*, *I. boryana* (Fig. 5A), *I. engelmannii* e *I. piedmontana*; es triangular en *I. andina* (Fig. 5O), *I. durieui*, *I. histrix*, *I. melanopoda* (Fig. 5D), *I. malinverniana* (Fig. 5G); triangular a plano-convexa en *I. anatolica*, *I. howellii*, *I. killipii*, *I. novogranadensis* (Fig. 5L), *I. nuttallii*, *I. setacea* e *I. storkii* (Fig. 5H) y es subcuadrangular a subcircular en *I. boliviensis* (Fig. 5Q), *I. brochonii*, *I. echinospora*, *I. lacustris*, *I. lechleri*, *I. longissima*, *I. orcuttii*, *I. velata* subsp. *velata* e *I. velata* subsp. *asturicensis*.

En corte transversal se observan los siguientes tejidos: epidermis, mesofilo y tejidos vasculares. La epidermis es uniestratificada y en especies terrestres o anfibias con secciones foliares más o menos triangulares, los márgenes foliares pueden estar claramente diferenciados y formados por células sobresalientes a papilosas. El mesofilo está constituido por clorénquima y presenta colénquima como tejido de sostén. El grado de desarrollo de ambos tejidos es variable en las especies y en todas se presentan cuatro cámaras aeríferas de posición más o menos simétrica en torno al haz vascular central. Las cámaras aeríferas tienen diafragmas transversales y presentan una endodermis.

Epidermis. Está cutinizada y desarrolla una membrana cuticular de espesor variable. La membrana cuticular está separada de la pared celulósica, que es delgada, por una fina capa péctica, que sólo se aprecia con más de 400 aumentos. La cutícula, de poco espesor, está formada por cutina pura. En muchas especies está ornamentada con estrías o estrías y verrugas, que generalmente involucran sólo la porción más externa de la membrana cuticular. Las capas cuticulares están compuestas por cutina y celulosa, tienen mayor espesor que la cutícula y a veces participan parcialmente en la formación de las verrugas cuticulares.

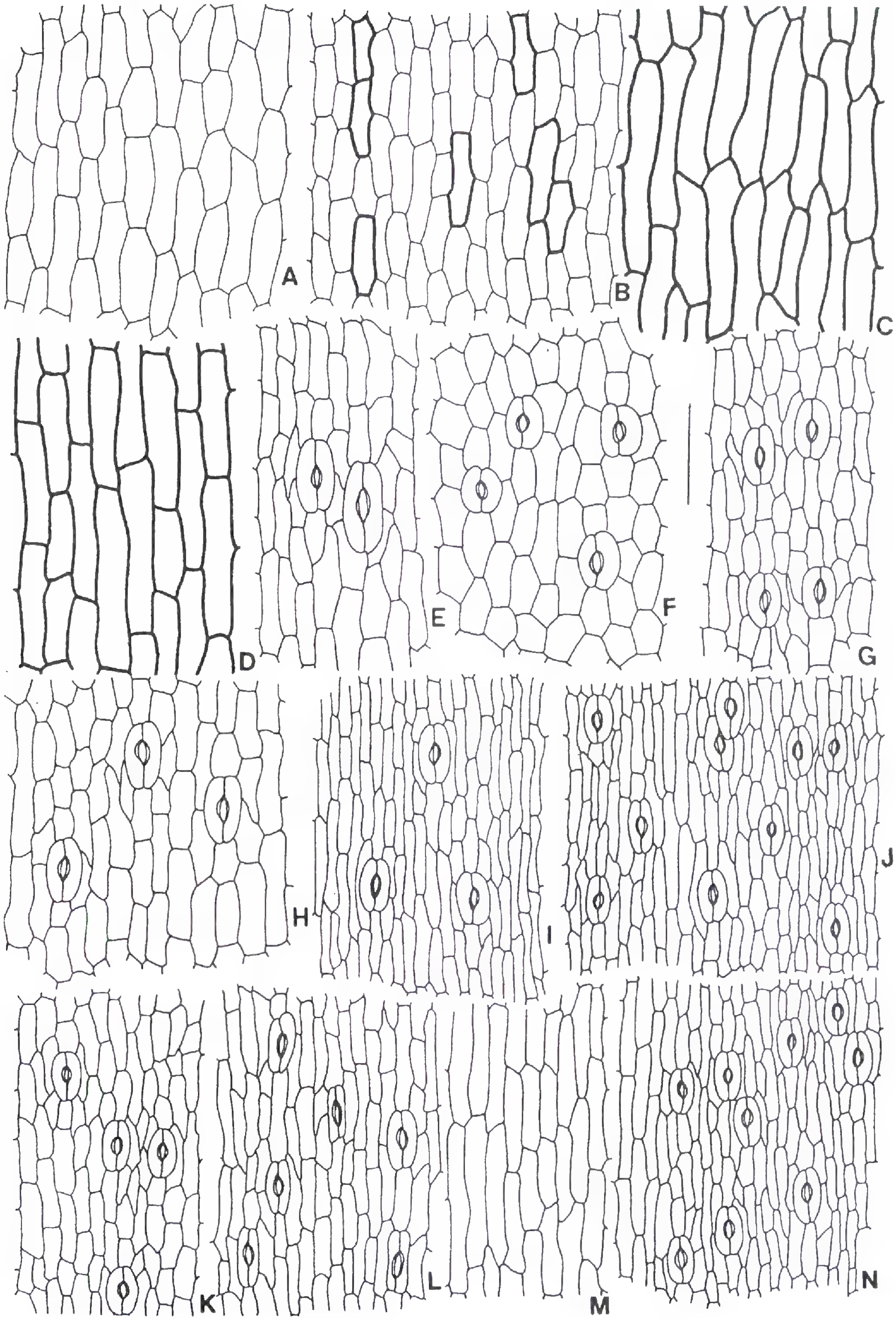

Figura 4. Modelos epidérmicos de los microfilos en especies de *Isoetes*. —A. *I. storkii*. —B. *I. lechleri*. —C. *I. andina*. D, E. *I. killipii*. —D. Parte media. —E. Área apical. —F. *I. borvana*. —G. *I. novo-granadensis*. —H. *I. malinverniana*. —I. *I. engelmannii*. —J. *I. piedmontana*. —K. *I. adspersa*. —L. *I. boliviensis*. M, N. *I. melanopoda*. —M. Área central de la parte media del microfilo. —N. Área marginal. El trazo grueso indica paredes muy engrosadas, tanto en grupos celulares aislados como en toda la epidermis. Escala: 100 µm.

Los mayores espesores de la membrana cuticular, de 5–8 µm, a veces más, son típicos de *Isoetes andina* (Figs. 4C, 5P), *I. killipii* (Figs. 4D, 5M) e *I. storkii* (Fig. 5N). Hay espesores intermedios, de 2–5 µm, en *I. boryana* (Fig. 5K), *I. histrix*, *I. lechleri* (Fig. 5F), *I. malinverniana* (Fig. 5E), *I. melanopoda* (Fig. 5B) e *I. novo-granadensis* (Fig. 5I). En las restantes especies se presentan espesores de 1–2 µm.

Las cuñas cuticulares sobresalientes se aprecian tanto en corte transversal como en superficie (Fig. 2B), mientras que las cuñas que se desarrollan hacia el interior de la epidermis sólo se aprecian en cortes transversales. Se han observado cuñas cuticulares internas profundas en *Isoetes malinverniana* (Fig. 5E), *I. melanopoda* (Fig. 5B) e *I. orcuttii*, todas ellas sin cuñas externas, pero también en algunas especies con cuñas externamente sobresalientes, como *I. boryana* (Fig. 5K), *I. novo-granadensis* (Fig. 5I) e *I. piedmontana* (Fig. 5J).

Sólo presentan márgenes bien definidos las especies con sección foliar más o menos triangular. En estas especies, las células epidérmicas marginales se disponen en 2–5 hileras longitudinalmente alargadas y con las paredes más engrosadas que las restantes epidérmicas; son papilosas en *Isoetes histrix* y prominentes pero no papilosas en las restantes especies. Cuando las secciones transversales foliares son cuadrangulares o casi circulares no se distinguen márgenes.

Mesofilo. Es relativamente simple y está formado por 1–5 capas de clorénquima de posición hipodérmica y 1–2 separando las cámaras aeríferas. La menor cantidad de capas aparece en especies con sección foliar subcuadrangular o subcircular. En microfilos de especies acuáticas con sección foliar subcuadrangular puede haber una sola capa de clorénquima.

Colénquima. En las especies estudiadas de *Isoetes* los microfilos presentan células con engrosamientos pectinados de tres tipos: localizados en toda la pared celular, localizados en paredes radiales y tangenciales externas o bien localizados hacia los espacios intercelulares. Todos estos engrosamientos se detectan mediante TBO, en cortes transversales y su naturaleza péctica se evidencia claramente por su reacción de color rosa ante dicho colorante.

El primer tipo de engrosamientos aparece en células que se agrupan en haces hipodérmicos y constituye lo que siempre se ha considerado colénquima en *Isoetes*. En general hay 3 haces adaxiales y 1 abaxial formados por más de 20 células en corte. Esta distribución puede variar en algunas especies por la formación de haces adicionales más pequeños, constituidos por menos de 10 células en corte. En *I. velata* subsp. *velata* se presentan hasta 30 o más haces pequeños y lo mismo se observó en *I. howellii* e *I. setacea*. El número de haces adicionales puede variar en la misma planta. El colénquima en haces se encuentra en los microfilos de *I. adspersa*, *I. anatolica*, *I. andina*, *I. boryana*, *I. durieui*, *I. echinospora*, *I. histrix*, *I. howellii*, *I. longissima*, *I. malinverniana*, *I. melanopoda*, *I. nuttallii*, *I. piedmontana*, *I. setacea*, *I. velata* subsp. *velata* e *I. velata* subsp. *asturicensis*. En *I. longissima* los haces son discontinuos y la distribución en 3 adaxiales y 1 abaxial puede verse modificada a lo largo del microfilo. En *I. echinospora* los haces de colénquima son extremadamente discontinuos y aparecen erráticamente, a veces uno solo, abaxial.

Los engrosamientos pectinados pueden ocupar la pared tangencial externa y parte de las paredes radiales de las células hipodérmicas del mesofilo. Así, se forma una capa más o menos continua de sostén por debajo de la epidermis, con paredes engrosadas a modo de un colénquima parcialmente laminar cuyas células no se agrupan en haces, como sucede en *Isoetes andina* e *I. storkii* (Fig. 5N), pero en *I. andina* se encuentra este tipo de engrosamiento en las células hipodérmicas situadas entre los 3 haces típicos de colénquima (Fig. 5O).

Los engrosamientos pectinados pueden desarrollarse también hacia los espacios intercelulares, a modo de un colénquima lacunar. Se sitúan en los espacios que dejan entre sí las células del mesofilo, en una o dos capas por debajo de la epidermis. Este tipo de colénquima se presenta en plantas jóvenes y adultas de *Isoetes lechleri* (Fig. 5C, F respectivamente), *I. melanopoda* (Fig. 5B), *I. orcuttii* e *I. piedmontana* (Fig. 5J) y puede estar presente aunque exista un colénquima formando haces típicos, como sucede en *I. melanopoda* e *I. piedmontana*.

No se presenta colénquima en *Isoetes boliviensis*, *I. brochonii*, *I. engelmannii*, *I. killipii*, *I. lacustris* e *I. novo-granadensis* (Tabla 1).

Diafragmas foliares y PIP. Los diafragmas foliares están formados por células globosas, lobuladas o estrelladas que se disponen dejando poros más o menos circulares, elípticos o triangulares. En la superficie y/o en las zonas de contacto de las lobulaciones o brazos de estas células se pueden presentar PIP. Los tipos de PIP descritos hasta el momento para *Isoetes* son verrugas, filamentos y conexiones pécticos. Se presentan verrugas en *I. adspersa*, *I. andina* (Fig. 6A), *I. durieui*, *I. engelmannii*, *I. killipii*, *I. lechleri*, *I. longissima*, *I. melanopoda*, *I. nuttallii*, *I. storkii*, *I. velata* subsp. *velata*, *I. velata* subsp. *asturicensis*; verrugas y filamentos en *I. brochonii*, *I. howellii*, *I. lacustris* e *I. setacea* (Fig. 6B)

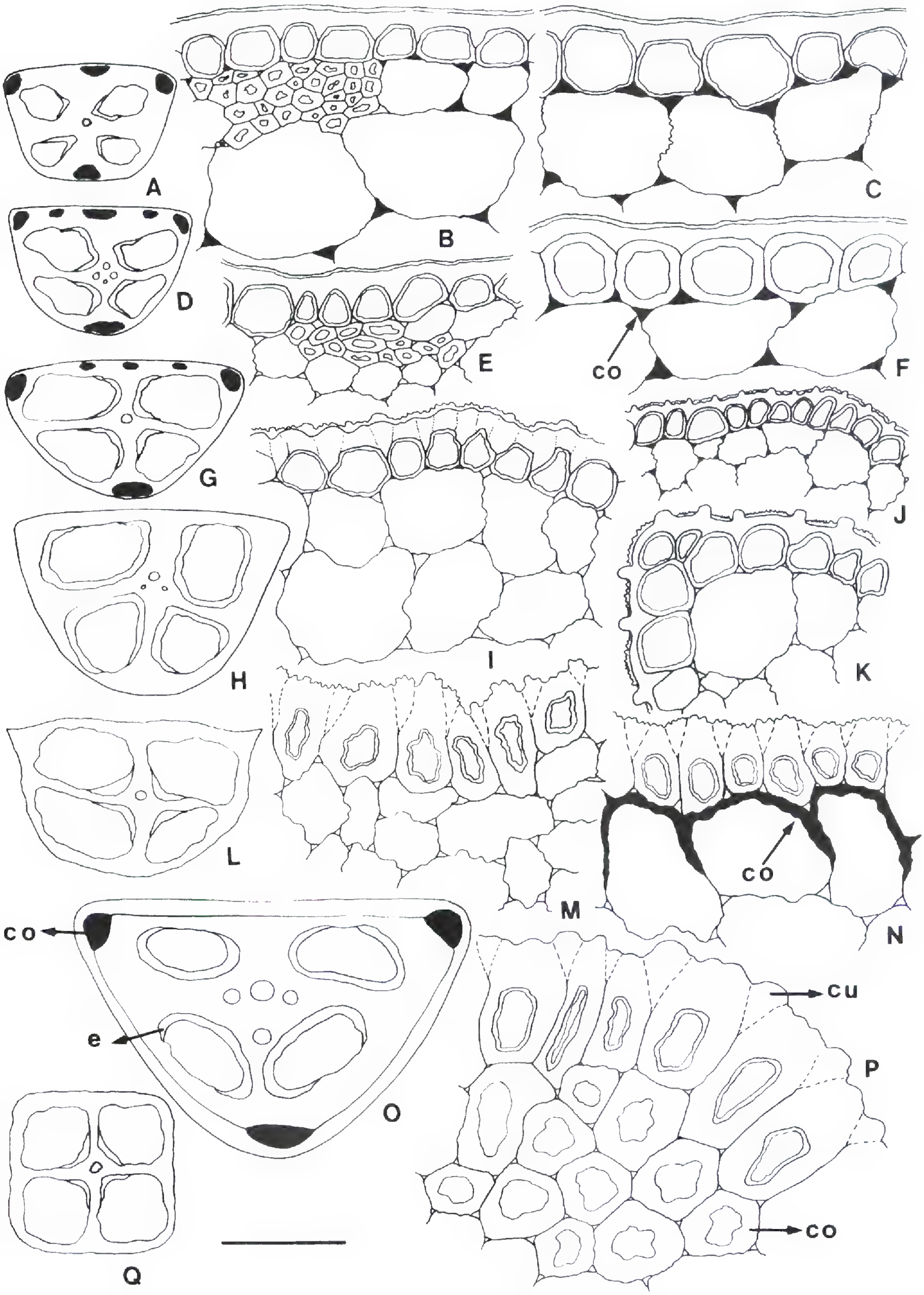

Figura 5. Esquemas y detalles de secciones foliares en especies de *Isoetes*. —A, K. *I. boryana*. —B, D. *I. melanopoda*. C, F. *I. lechleri*. —C. Plantas jóvenes. —F. Plantas maduras. —E, G. *I. malinverniana*. —H, N. *I. storkii*. —I, L. *I. novogranadensis*. —J. *I. piedmontana*. —M. *I. killipii*. —O, P. *I. andina*. —Q. *I. boliviensis* (similar en *I. lechleri*). co, colénquima; cu, cuñas cuticulares; e, endodermis externa. Escala: 1.5 mm en A, D, G, H, L, O, Q; 50 µm en B, C, E, F, I–K, M, N, P.

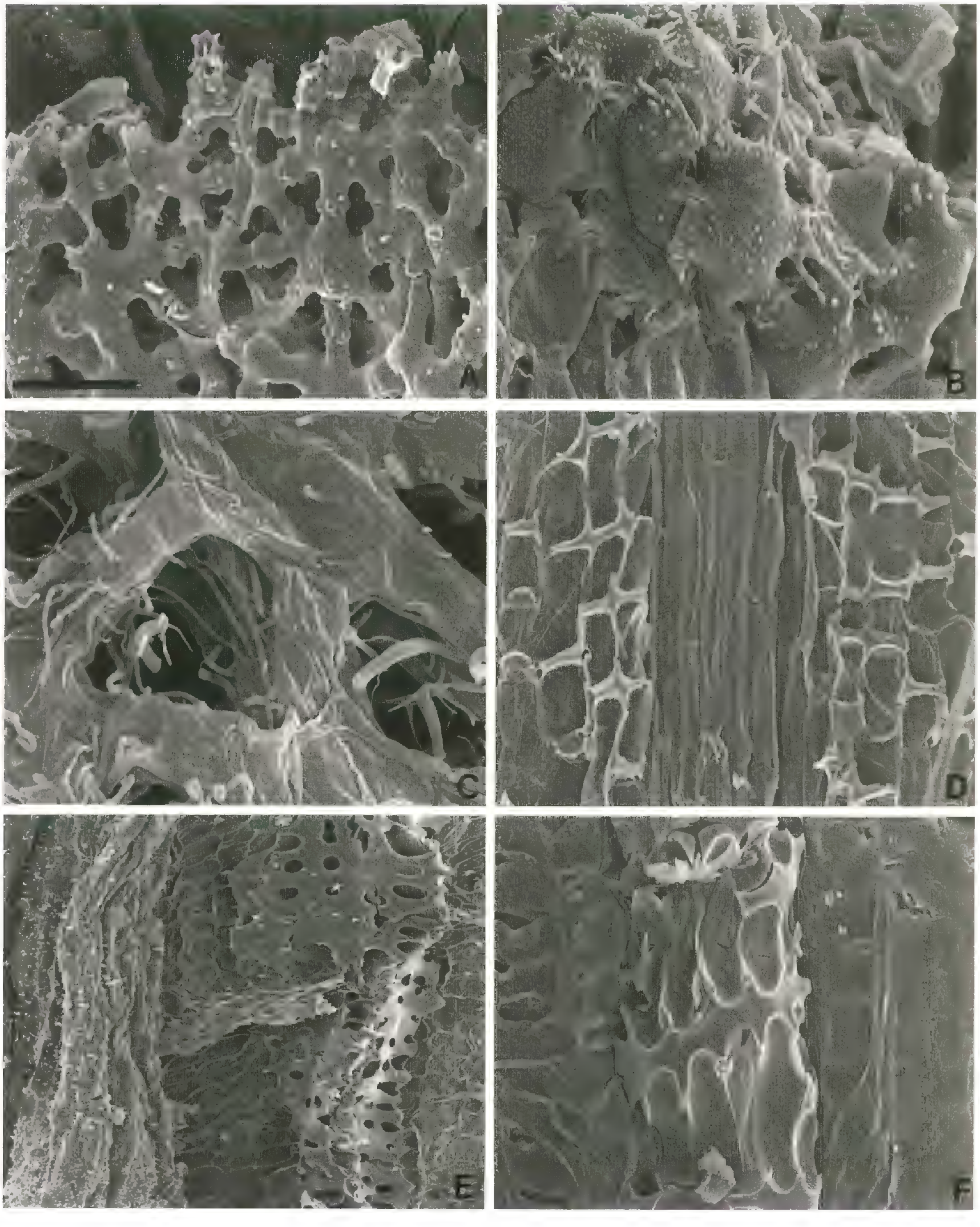

Figura 6. Protuberancias intercelulares pécticas y endodermis externa en especies de *Isoetes*. —A. *I. andina*, células estrelladas de un diafragma foliar con verrugas. —B. *I. setacea*, células lobuladas de un diafragma foliar con verrugas y filamentos. —C. *I. anatolica*, células estrelladas de un diafragma foliar con filamentos y conexiones. —D. *I. velata*, corte longitudinal de microfilo mostrando dos cámaras aeríferas con endodermis externa parcial y células con engrosamientos en U, separadas por el área de la vena media. —E. *I. storkii*, corte longitudinal de microfilo mostrando una cámara aerífera con endodermis externa completa y células con engrosamientos en O. —F. *I. anatolica*, corte longitudinal de microfilo mostrando una cámara aerífera con endodermis externa parcial y células con engrosamientos en O. Escala: 80 µm en A, D, F; 30 µm en B; 15 µm en C; 250 µm en E.

y se encuentran filamentos y conexiones pécticos en *I. anatolica* (Fig. 6C) e *I. malinverniana*. No se observaron PIP en *I. boliviensis*, *I. boryana*, *I. echinospora*, *I. histrix*, *I. novo-granadensis* e *I. orcuttii* (Tabla 1). *Isoetes histrix* presenta ocasionales verrugas pécticas en las paredes de la epidermis que dan hacia las cámaras aeríferas y las especies con verrugas en las células de los diafragmas pueden presentarlas igualmente en las paredes de las células del mesofilo que dan hacia esas cámaras. Las PIP se detectan fácilmente mediante el TBO en cortes transversales o arrancados de mesofilo; su naturaleza péctica se pone de manifiesto por su reacción de color rosa intenso con el colorante mencionado.

Endodermis. En todas las especies estudiadas se presentan dos endodermis foliares: una situada en la zona del haz vascular, la endodermis interna o de los canales intrastelares y otra en la porción del mesofilo que da hacia las cámaras aeríferas, la endodermis externa o de las cámaras. Ambas tienen desarrollo variable en relación con el grado de engrosamiento de sus paredes. La endodermis de las cámaras es parcial, es decir está restringida a la porción curva de la cámara que limita directamente con el área del haz vascular, en todas las especies estudiadas, con excepción de *Isoetes andina*, que presenta una endodermis completa o casi completa. Los engrosamientos de las células endodérmicas son de dos tipos: en U o en O. Los engrosamientos en U se presentan en las endodermis de las cámaras (Fig. 6D) y en la de los canales intrastelares de las especies estudiadas, aunque en sus primeras etapas esta endodermis tiene bandas de Caspary. Los engrosamientos en O solamente se observaron en las endodermis de las cámaras de *I. anatolica* (Fig. 6F), *I. andina* e *I. storkii* (Fig. 6E), en las que pueden ser tan gruesos que se desprenden trozos de pared hacia las cámaras mismas cuando se efectúan cortes transversales.

Area del haz vascular. En el área del haz el tejido vascular es relativamente escaso y está reducido a una venilla media constituida por xilema y floema, rodeada de tejido parenquimático variablemente compacto. El protoxilema se reabsorbe y deja uno o más canales intrastelares en los cuales se desarrolla la endodermis interna con bandas de Caspary. La mayoría de las especies estudiadas presentan un canal intrastelar. Se observaron 4 canales en *Isoetes andina* e *I. melanopoda*, 3 en *I. durieui*, *I. howellii*, *I. setacea* e *I. storkii* mientras que en *I. histrix* el número es variable entre 1 y 5.

LÍGULA Y LABIO

La lígula se encuentra presente en todos los microfilos y es un órgano secretor de mucílago. Se forma tempranamente y alcanza rápidamente su máximo desarrollo; se ha observado que las lígulas de microfilos de 1–2 cm de *Isoetes brochonii*, *I. durieui*, *I. echinospora* e *I. lacustris* tienen ya su tamaño final.

La lígula tiene forma de hoja, de contorno ovado o lanceolado. Lígulas lanceoladas se encontraron en *Isoetes anatolica* (Fig. 7D), *I. andina* (Fig. 7M), *I. durieui* (Fig. 7L), *I. echinospora* (Fig. 7H), *I. histrix* (Fig. 7B), *I. killipii*, *I. malinverniana*, *I. melanopoda* (Fig. 7O), *I. novo-granadensis* (Fig. 7G), *I. nuttallii*, *I. orcuttii* (Fig. 17M), *I. setacea* (Fig. 7A) e *I. velata* (Fig. 7K). Lígulas ovadas aparecen en *I. adspersa*, *I. boliviensis*, *I. boryana* (Fig. 8K), *I. brochonii* (Fig. 8G), *I. engelmannii*, *I. howellii*, *I. lacustris* (Fig. 8J), *I. longissima* (Fig. 8D), *I. piedmontana* (Fig. 8L) e *I. storkii* (Fig. 8A). Finalmente, la lígula es anchamente ovada a casi semicircular en *I. lechleri* (Fig. 8O).

El área marginal de las lígulas es uniestratificada, pero su anchura varía en las especies. Esta área puede ser activamente secretora, función que se pone de manifiesto mediante el TBO por la reacción de color verde de los núcleos, su posición central en las células y por el color rosa intenso de las paredes de éstas.

Se ha observado variación en la duración y localización de la función secretora de la lígula. En microfilos maduros de algunas especies, las lígulas tienen un área marginal uniestratificada que permanecería secretora hasta la madurez, mientras que las de otras especies tienen una zona marginal que ya no es secretora. El cese de la función secretora se observa claramente por la posición parietal de los núcleos de las células de la porción marginal de la lígula, cuyas paredes se impregnan de taninos. En otras especies, la función secretora de la zona marginal es más bien efímera y en microfilos maduros sus células aparecen vacías y se esclerosan por completo. En todos los casos y aún en las lígulas con una zona marginal papirácea esclerosada, las células del ápice conservan su función secretora. El ápice de las lígulas suele estar formado por células largas, similares a pelos (Figs. 7C, 8E, H) o células agudas pero no piliformes (Fig. 8B). El margen puede presentar células piliformes (Figs. 7J, 8I, M) o bien células apenas prominentes, como dentículos, más o menos largos, con paredes primarias (Figs. 7E, F, I, 8C, F) o esclerosadas (Figs. 7N, 8N).

Lígulas permanentemente secretoras, con el área de secreción más o menos extensa, apical y marginal, se encontraron en *Isoetes anatolica* (Fig. 7F), *I. boryana* (Fig. 8E, F), *I. histrix* (Fig. 7C), *I. howellii*, *I. lacustris* (Fig. 8M), *I. setacea* (Fig. 7J) e *I. velata* (Fig. 7I). Las lígulas de *I. andina* (Fig. 7N), *I. brochonii* (Fig. 8N) e *I. killipii* se esclerosan tempranamente y sólo

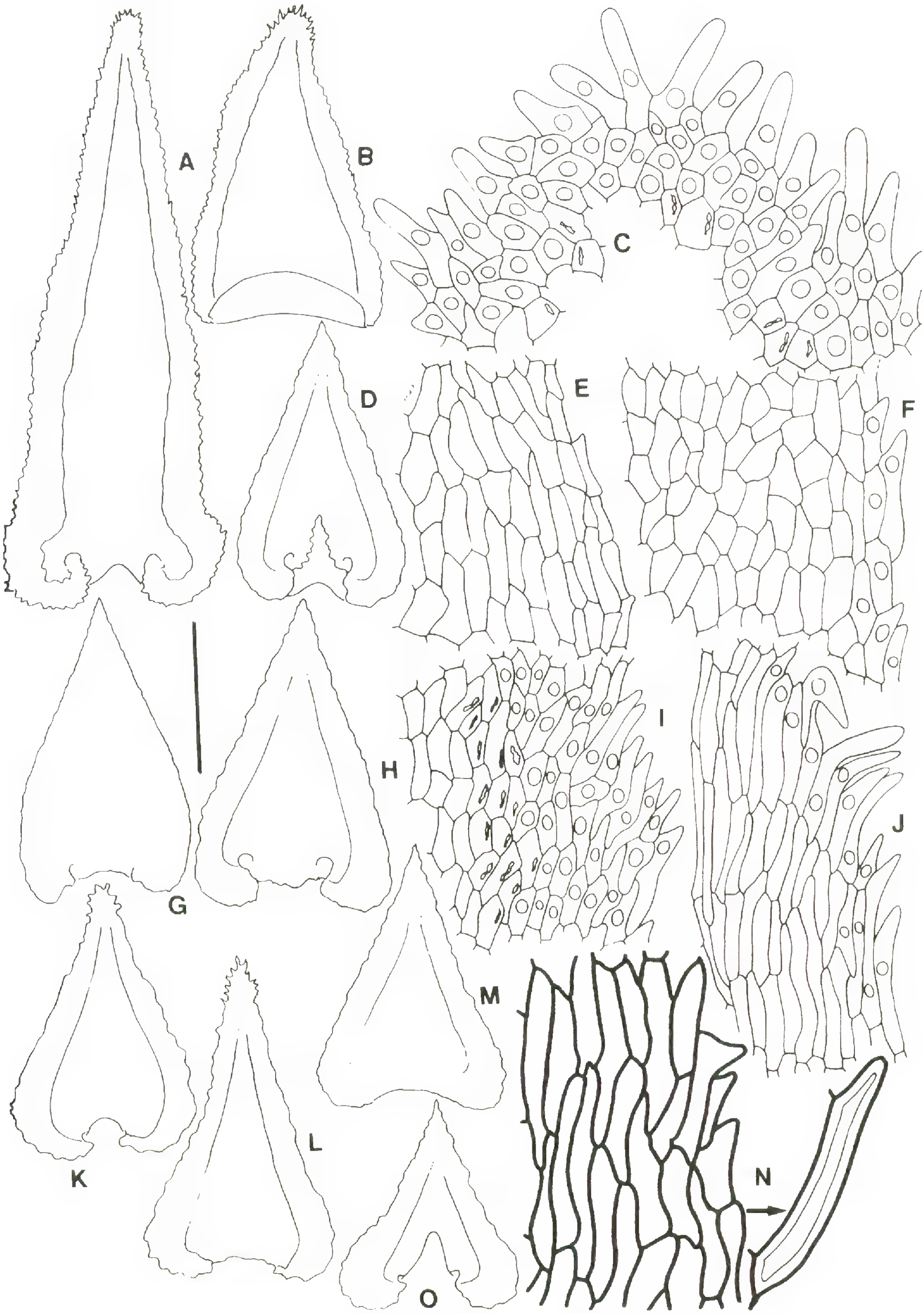

Figura 7. Contornos y modelos epidérmicos de lígulas lanceoladas en especies de *Isoetes*. —A, J. *I. setacea*. —B, C. *I. hystrix*. —D, F. *I. anatolica*. —E, O. *I. melanopoda*. —G. *I. novo-granadensis*. —H. *I. echinospora*. —I, K. *I. velata*. —L. *I. durieui*. —M, N. *I. andina*. Las células con núcleos ilustrados corresponden al área secretora; los núcleos contraídos indican el área donde la secreción cesa; si no hay función secretora no se han ilustrado núcleos. El trazo grueso en las paredes indica células lignificadas. Escala: 3 mm en A; 1 mm en B, D, G, H, K, L, M, O; 150 µm en C, E, F, I, J; 50 µm en N.

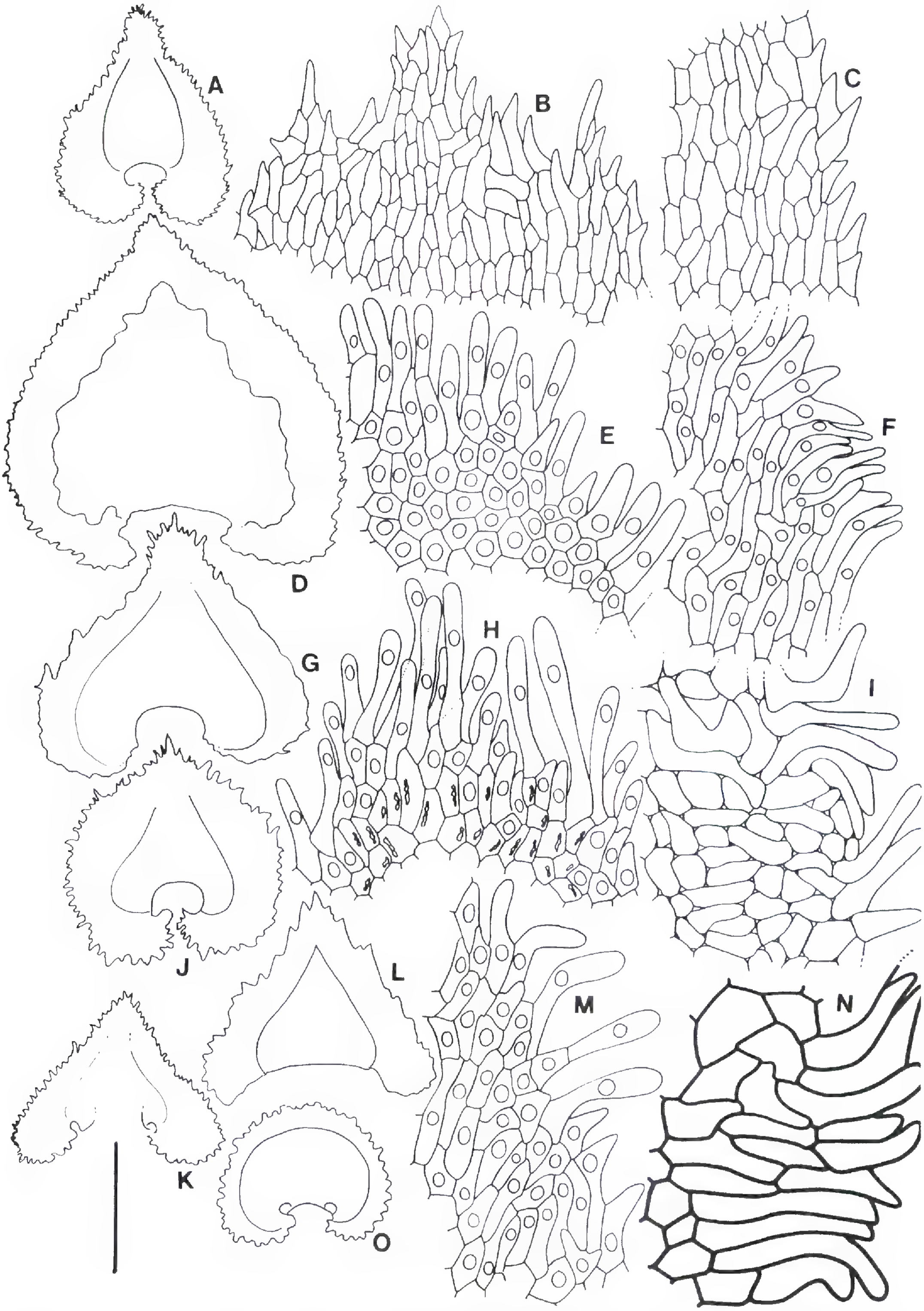

Figura 8. Contornos y modelos epidérmicos de lígulas ovadas en especies de *Isoetes*. —A, I. *I. storkii*. —B, C, L. *I. piedmontana*. —D. *I. longissima*. —E, F, K. *I. boryana*. —G, H, N. *I. brochonii*. —J, M. *I. lacustris*. —O. *I. lechleri*. Las células con núcleos ilustrados corresponden al área secretora; los núcleos contraídos indican el área donde la secreción cesa; si no hay función secretora no se han ilustrado núcleos. El trazo grueso en las paredes indica células lignificadas. Escala: 1 mm en A, D, G, J, K, L, O; 150 µm en B, C, E, F, H, I, M, N.

conservan ápices secretores. En las restantes especies la secreción parece cesar en microfilos adultos.

El área central de las lígulas es pluriestratificada, con 2–3 capas celulares de espesor o, a veces, algo más, con el aspecto de un mesofilo simple (Fig. 9A–F). En el caso de *Isoetes histrix* (Fig. 9A), la epidermis de la lígula se destaca por sus células papilosas en el área central pluriestratificada, un rasgo que no se ha observado en otras especies.

El labio es un órgano carnoso, más bien rígido, resistente, situado por debajo de la lígula, con un desarrollo y forma que pueden variar en la misma planta. Aquí sólo se ha estudiado en *Isoetes setacea* y se ha podido comprobar que es una estructura amilácea, con una epidermis tempranamente cutinizada, casi siempre formada por células isodiamétricas con paredes muy engrosadas dispuestas con modelo poligonal y presenta una organización interna muy sencilla (Fig. 9H). Las células de la epidermis y las internas almacenan almidón de granos simples, más o menos esféricos, con un hilo excéntrico. Estos granos (Fig. 9I) son similares a los que provienen de los amiloplastos del cormo pero son notablemente más grandes. Se acumulan en todas las células del labio, incluidas las epidérmicas, con excepción de las que ocupan una posición abaxial basal (Fig. 9G).

VELO

El velo y el labio son connatos, pero su desarrollo relativo puede variar. El velo se extiende basiscópicamente en forma de un tejido 2–3 estratificado, que puede cubrir totalmente o parcialmente el esporangio y se continúa lateralmente en el tejido del ala. Las células de las capas hipodérmicas pueden contener abundante almidón pero en ellas los granos tienen un tamaño similar a los del cormo.

Todas las especies estudiadas tienen velo excepto *Isoetes anatolica*, *I. malinverniana* e *I. setacea*. El grado de desarrollo del velo ha sido utilizado como rasgo específico, pero aquí se ha visto que su extensión y la consecuente cobertura del esporangio puede variar en microfilos de la misma planta. En *I. histrix* el velo suele ser completo pero ocasionalmente falta en macrosporofilos; en *I. andina* es muy corto pero también puede estar ocasionalmente ausente; en *I. velata* el velo suele ser completo en los macrosporangios y corto o incompleto en los microsporangios.

El modelo epidérmico de la cara adaxial del velo muestra variación específica. Los modelos se caracterizan por las paredes celulares sinuosas o rectas (modelos sinuosos o poligonales), la relación l/a celular y los tamaños celulares. Las células tienen pared primaria celulósica débilmente cutinizada y en algunas especies, como *Isoetes adspersa*, *I. brochonii* e *I. echinospora*, presentan taninos.

Modelos sinuosos se presentan en *Isoetes lechleri* (Fig. 10B), *I. longissima*, *I. nuttallii*, *I. orcuttii*, *I. piedmontana* (Fig. 10A), *I. velata* subsp. *velata* (Fig. 10C) e *I. velata* subsp. *asturicensis*; subsinuosos en *I. adspersa* (Fig. 10H), *I. boliviensis* (Fig. 10E), *I. boryana* (Fig. 10F), *I. echinospora* (Fig. 10D) e *I. melanopoda* (Fig. 10G), y finalmente, modelos poligonales, en *I. andina* (Fig. 11G), *I. brochonii* (Fig. 11B), *I. durieui* (Fig. 11H), *I. histrix* (Fig. 11F), *I. howellii*, *I. killipii* (Fig. 11E), *I. lacustris* (Fig. 11C), *I. novo-granadensis* (Fig. 11A) e *I. storkii* (Fig. 11D).

Las relaciones l/a celulares son 1:1 en *Isoetes boliviensis* e *I. piedmontana*, 2:1 en *I. brochonii*, *I. durieui*, *I. killipii*, *I. lacustris*, *I. novo-granadensis* e *I. storkii*. Relaciones l/a celulares 3:1 son característicos de *I. andina*, *I. echinospora*, *I. histrix*, *I. lechleri* e *I. orcuttii*, 4:1 en *I. boryana*, *I. howellii*, *I. nuttallii* e *I. velata*. Las relaciones l/a celulares más altas, 5–6:1 y 12–13:1, se encontraron en *I. melanopoda* e *I. adspersa* respectivamente (Tabla 1).

PARED ADAXIAL DE LOS ESPORANGIOS

La epidermis adaxial del esporangio puede estar formada por distintos tipos celulares. En ciertas especies está constituida por células que sólo desarrollan pared primaria celulósica débilmente cutinizada, que da reacción de color rosa con el TBO y sin inclusiones detectables por los métodos microquímicos aquí utilizados. En otras especies, algunas de las células epidérmicas contienen taninos, los cuales se detectan porque dan reacción de color verde claro con el TBO, que se atribuye a la presencia de polifenoles. Por último, en otras especies, la epidermis está formada por células con pared secundaria lignificada. La lignina se pone en evidencia por su reacción de color verde intenso hasta azul brillante con el TBO. El color verde de las paredes lignificadas también se debe a polifenoles, y es similar en ligninas y taninos. La fluorescencia verdosa que emiten exclusivamente las paredes lignificadas permite discriminar entre unas y otros.

Modelos epidérmicos de la pared adaxial del esporangio. Los modelos epidérmicos formados por células con pared primaria débilmente cutinizada, dispuestas en grupos definidos, como un parqué y con las células de los distintos grupos contactando entre sí por extremos romos se presentan en *Isoetes boliviensis* (Fig. 12D), *I. boryana* (Fig. 12C), *I. lacustris* (Fig. 13A), *I. longissima* (Fig. 12B), *I. melanopoda* (Fig. 13B), *I. novo-granadensis* (Fig. 12F), *I. nuttallii*, *I. orcuttii*, *I. storkii* (Fig. 12E) e *I. velata* (Fig. 12A).

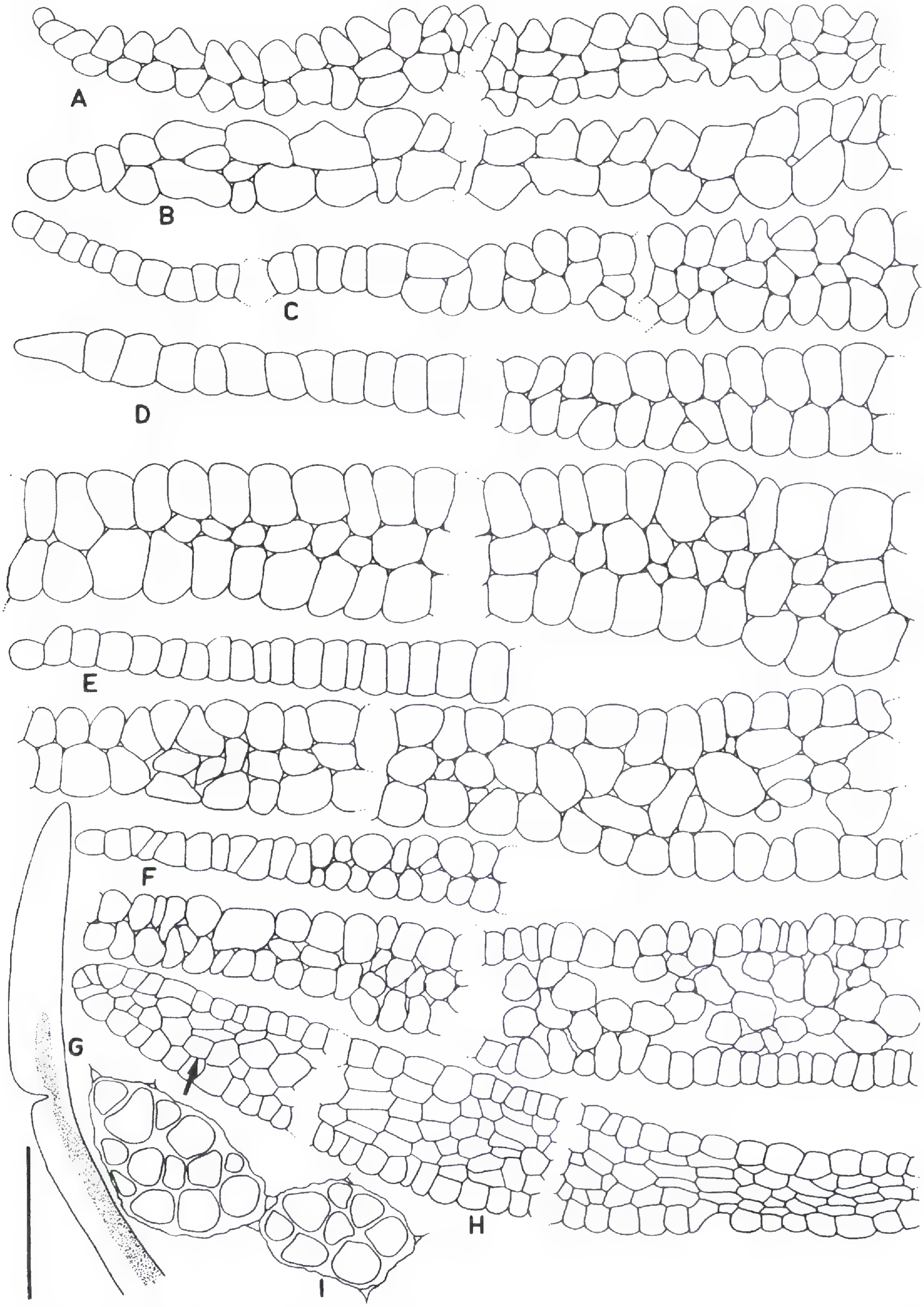

Figura 9. Secciones tranversales de lígulas y labio en especies de *Isoetes*. A–F. Lígulas. —A. *I. histrix*, lígula 3-estratificada con epidermis abaxial y adaxial papilosa. —B. *I. andina*, lígula 2-estratificada. —C. *I. setacea*, lígula 3-estratificada con epidermis abaxial y adaxial subpapilosas. D–F. Lígulas pluriestratificadas, de sección similar a un mesofilo sencillo. —D. *I. storkii*. —E. *I. lechleri*. —F. *I. velata*. G–I. Labio de *I. setacea*. —G. Sección longitudinal del labio completo. —H. Detalle de la anatomía. —I. Células con granos de almidón. Sombreado, área no amilácea del labio. Escala: 150 µm en A–F, H; 1 mm en G; 75 µm en I.

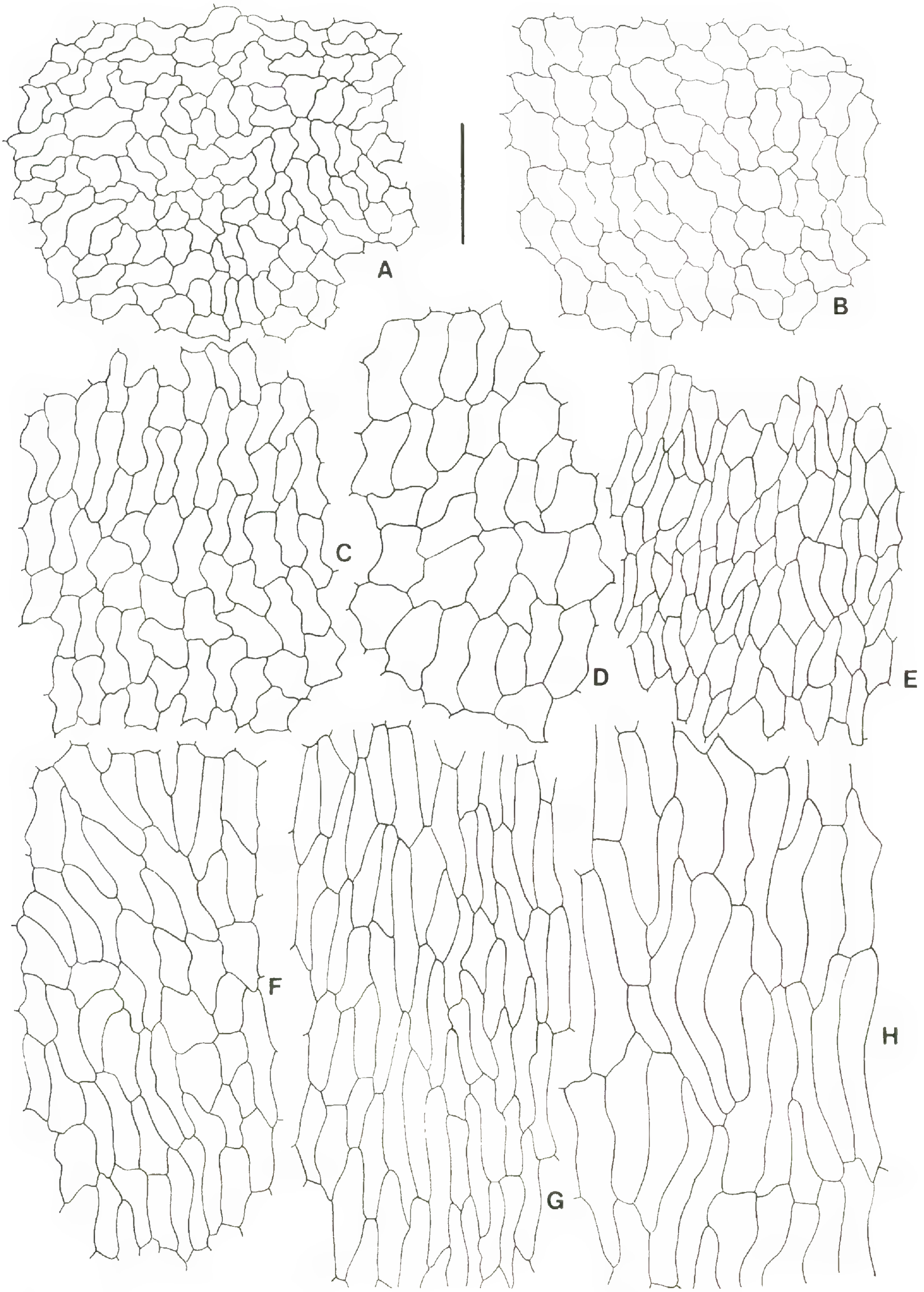

Figura 10. Modelos epidérmicos del velo en especies de *Isoetes*. —A. *I. piedmontana*. —B. *I. lechleri*. —C. *I. velata*. —D. *I. echinospora*. —E. *I. boliviensis*. —F. *I. boryana* (similar en *I. longissima*). —G. *I. melanopoda*. —H. *I. adspersa*. Escala: 100 µm.

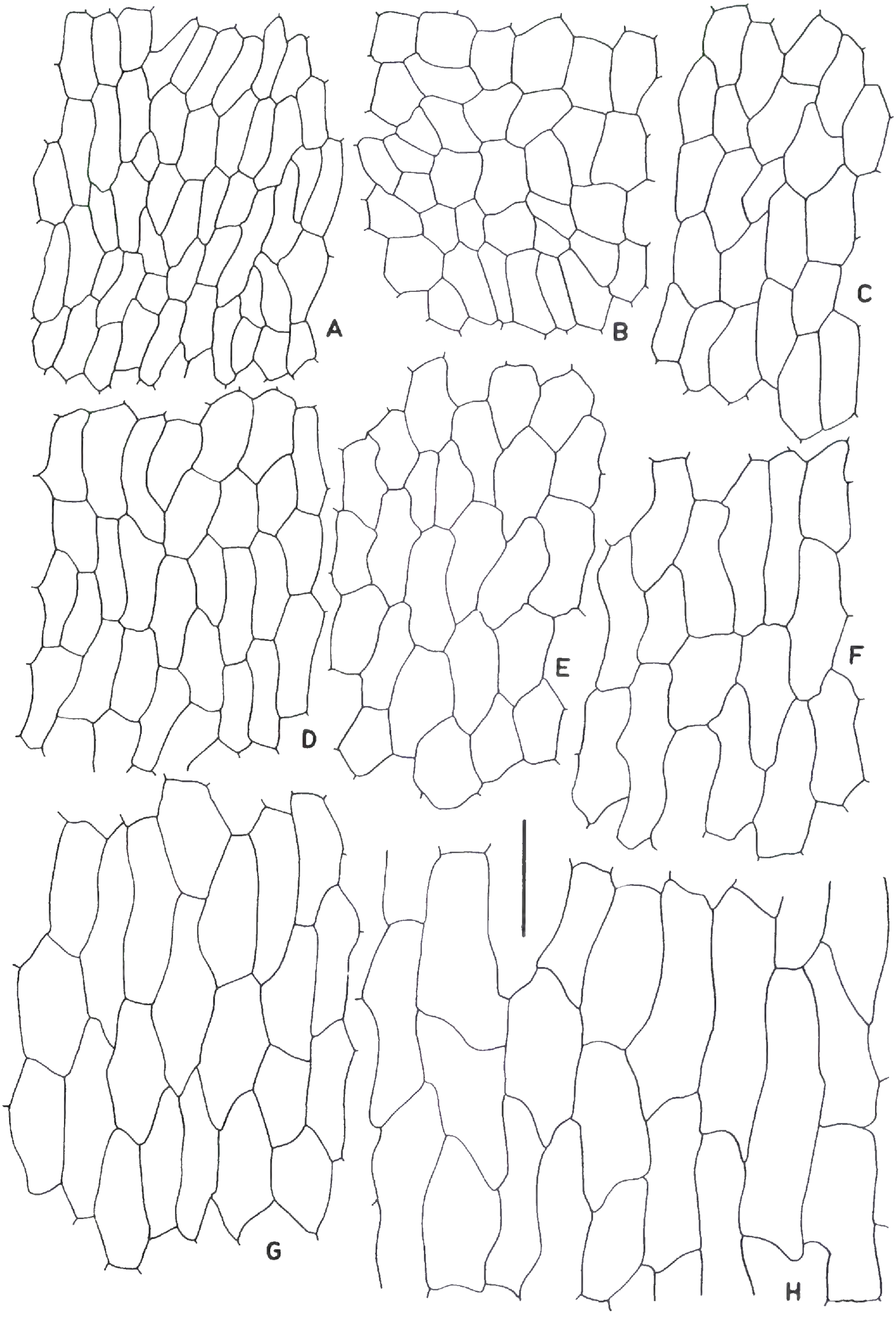

Figura 11. Modelos epidérmicos del velo en especies de *Isoetes*. —A. *I. novo-granadensis*. —B. *I. brochonii*. —C. *I. lacustris*. —D. *I. storkii*. —E. *I. killipii*. —F. *I. histrix*. —G. *I. andina*. —H. *I. durieui*. Escala: 100 µm.

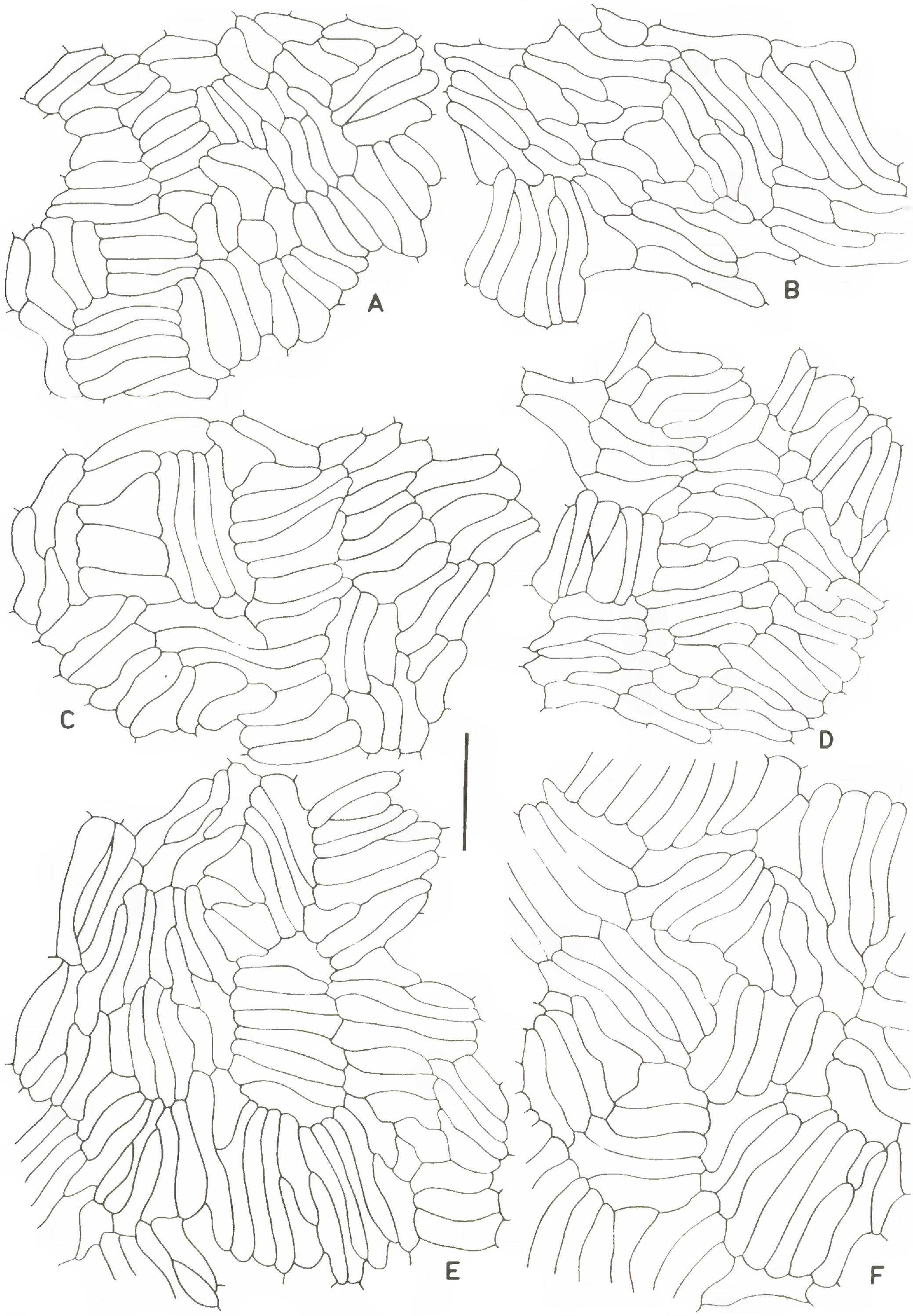

Figura 12. Modelos epidérmicos de la pared adaxial del esporangio en especies de *Isoetes*. Modelo en parqué, células con pared primaria cutinizada. —A. *I. relata*. —B. *I. longissima*. —C. *I. boryana*. —D. *I. boliviensis*. —E. *I. storkii*. —F. *I. novo-granadensis*. Escala: 100 μm.

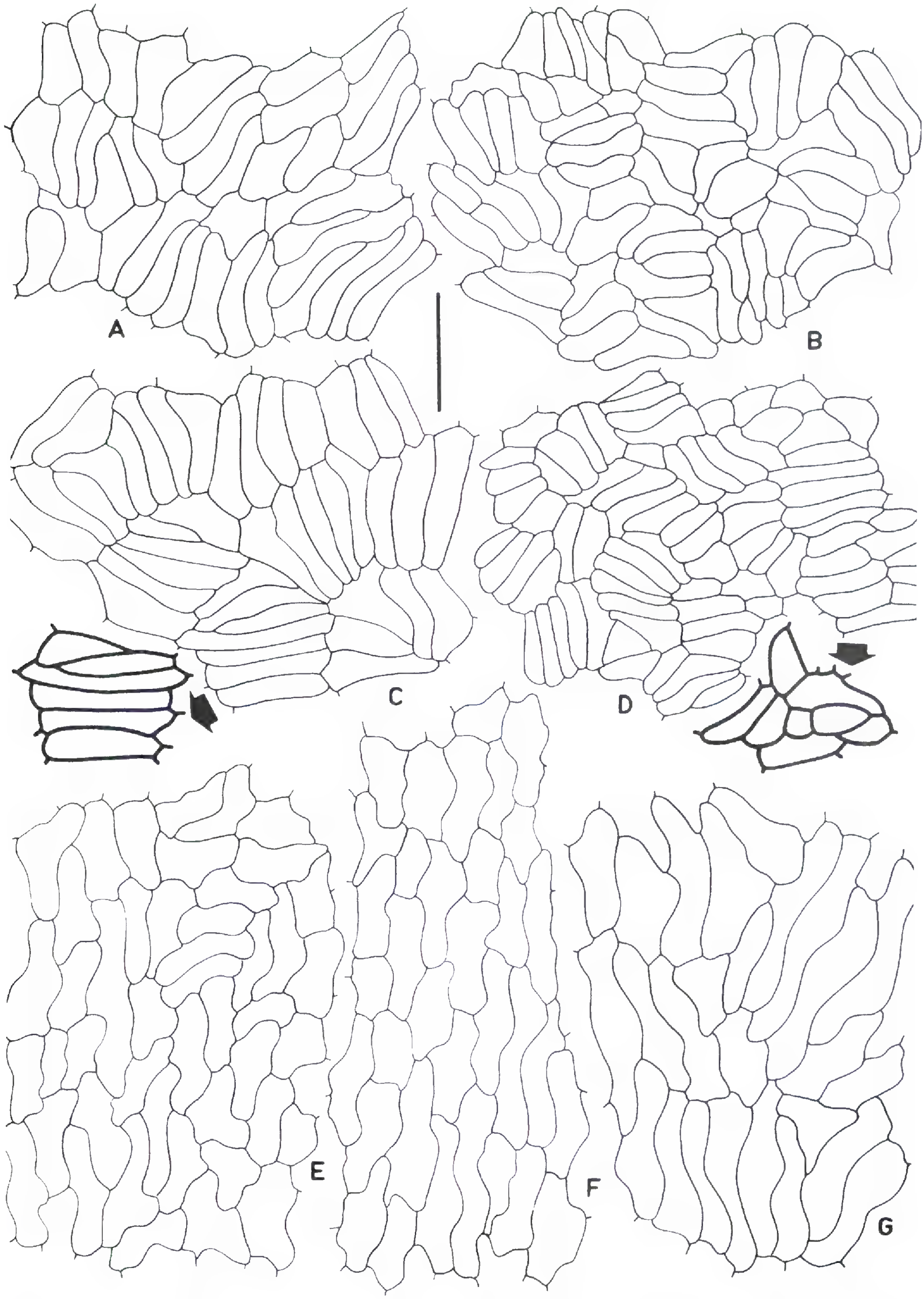

Figura 13. Modelos epidérmicos de la pared adaxial del esporangio en especies de *Isoetes*. A, B. Modelo en parqué, células con pared primaria cutinizada. —A. *I. lacustris*. —B. *I. melanopoda*. —C. Modelo en parqué, células con pared secundaria lignificada, en *I. andina*. —D. Modelo en parqué, células con pared engrosada (cutinizada) no lignificada, en *I. lechleri*. E–G. Modelo subsinuoso no en parqué, células con pared primaria cutinizada. —E. *I. malinverniana*. —F. *I. anatolica*. —G. *I. killipii*. Flechas, detalle de engrosamiento uniforme de la pared celular. Escala: 100 µm.

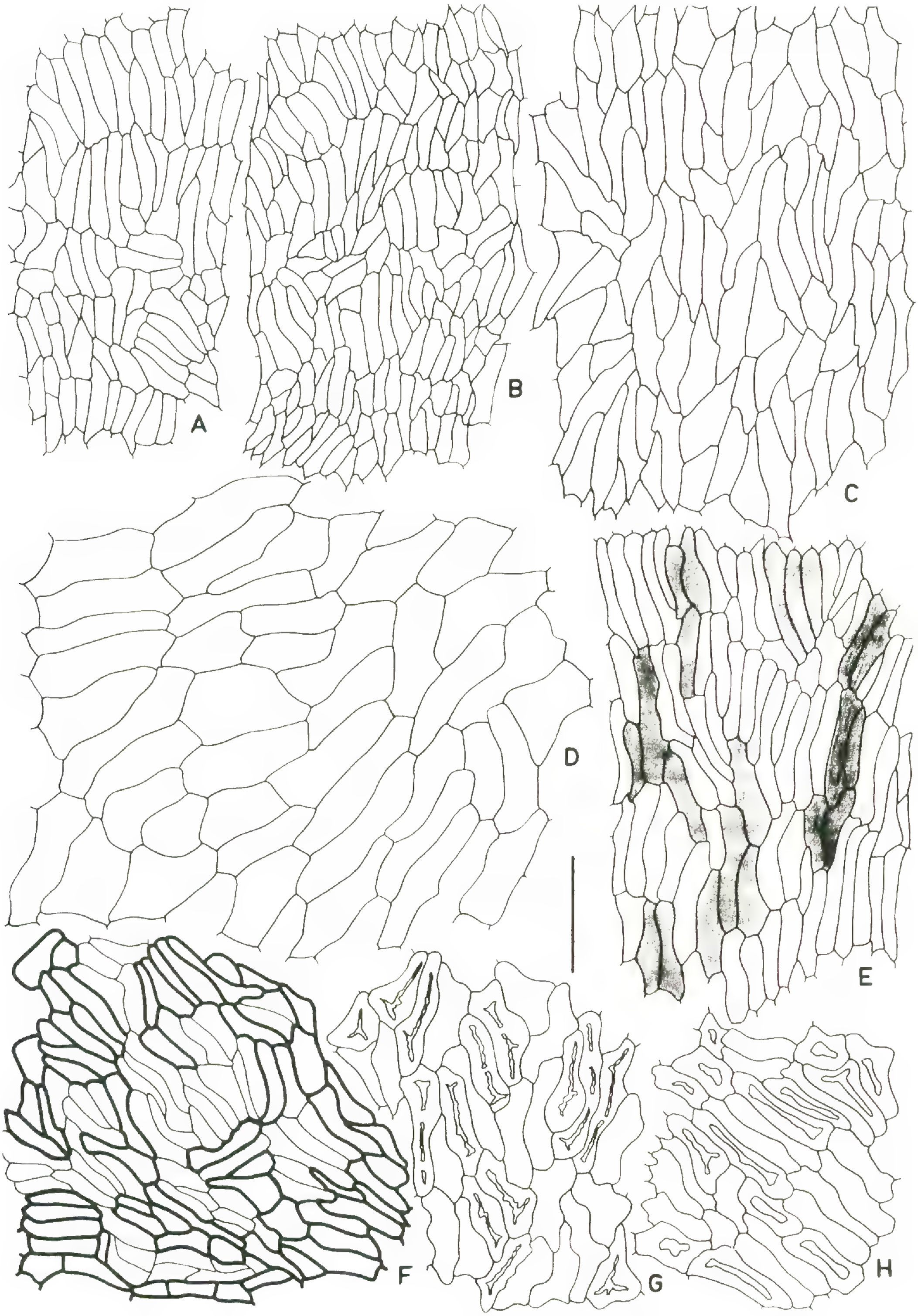

Figura 11. Modelos epidérmicos de la pared adaxial del esporangio en especies de *Isoetes*. A–C. Modelos en parqué, células con pared primaria cutinizada, angulosas. —A. *I. engelmannii*. —B. *I. echinospora*. —C. *I. brochonii*. —D. Modelo poligonal, células con pared primaria cutinizada en *I. durieui*. —E. Modelo en parqué, células con pared primaria tanífera (áreas oscuras), en *I. adspersa*. F–H. Modelo en parqué, células con pared primaria cutinizada y células con pared secundaria lignificada. —F. *I. piedmontana*. —G. *I. histrix*. —H. *I. setacea*. Escala: 100 μm.

Tabla 2. Caracteres de la pared adaxial del esporangio en especies de *Isoetes*.

Con grupos celulares definidos, "en parqué"	Paredes primarias débilmente cutinizadas	Paredes celulósicas delgadas	Los grupos celulares contactan por paredes curvas	*I. boliviensis, I. boryana, I. lacustris, I. longissima, I. melanopoda, I. novo-granadensis, I. nuttallii, I. orcuttii, I. storkii, I. velata*
			Los grupos celulares contactan por paredes agudas	*I. brochonii, I. echinospora, I. engelmannii*
		Paredes celulósicas engrosadas	Células con taninos	*I. adspersa, I. howellii*
			Células sin taninos	*I. lechleri*
	Paredes lignificadas en todas las células o en parches celulares	Todas las células lignificadas		*I. andina*
		Sólo grupos de células lignificadas, formando parches		*I. histrix, I. piedmontana, I. setacea*
Sin grupos celulares definidos	Modelo epidérmico poligonal			*I. durieui*
	Modelo epidérmico subsinuoso			*I. anatolica, I. killipii, I. malinverniana*

En *I. lechleri* las paredes son más gruesas que en las otras especies (Fig. 13D). Este modelo se presenta también en *I. adspersa* (Fig. 14E) e *I. howellii*, pero en estas especies hay grupos celulares que están coloreados de castaño por la presencia de taninos. Las células de los distintos grupos contactan entre sí por extremos agudos en *I. brochonii* (Fig. 14C), *I. echinospora* (Fig. 14B) e *I. engelmannii* (Fig. 14A).

Los modelos epidérmicos formados íntegramente por células esclerosadas, dispuestas en grupos definidos, como un parqué, con las células de los distintos grupos contactando entre sí por extremos romos se presentan en *Isoetes andina* (Fig. 13C).

Los modelos epidérmicos formados por grupos definidos de células, no esclerosadas y esclerosadas, como un parqué, se han encontrado en *Isoetes histrix* (Fig. 14G), *I. piedmontana* (Fig. 14F) e *I. setacea* (Fig. 14H).

Las epidermis formadas por células con paredes primarias débilmente cutinizadas, sin esclerosar, dispuestas con modelo subsinuoso, no en grupos definidos como un parqué, se encontraron en *Isoetes anatolica* (Fig. 13F), *I. killipii* (Fig. 13G) e *I. malinverniana* (Fig. 13E). Epidermis de este tipo pero con modelo poligonal y con células notablemente más grandes que en las demás especies, se presenta en *I. durieui* (Fig. 14D).

Si la epidermis adaxial del esporangio está formada solamente por células con pared primaria, la pared se verá externamente sin color salvo que algunas de las células epidérmicas contengan taninos, como es el caso de *Isoetes adspersa* e *I. howellii*. Cuando todas las células o grupos de ellas están lignificadas, los esporangios pueden aparecer uniformemente coloreados (*I. andina*), veteados de castaño (*I. piedmontana*) o no presentar externamente color a pesar de tener paredes lignificadas (*I. histrix* e *I. setacea*).

El color externo no indica ni la naturaleza de las paredes ni las eventuales inclusiones presentes y su ausencia tampoco implica que los modelos epidérmicos coincidan. El método de coloración empleado aquí permite determinar los modelos y la naturaleza de las paredes de las células que los forman (taninos o paredes secundarias lignificadas). Los taninos están ampliamente difundidos en *Isoetes*, se han detectado en las células de la epidermis de los microfilos, en los filopodios, escamas foliares, epidermis de algunos velos y esporangios. En los esporangios con células lignificadas que tienen color castaño externamente visible, es posible que el color se deba a una mezcla de sustancias en las paredes y la coloración con TBO puede coincidir, porque los polifenoles son precursores de taninos y de lignina. Como los taninos no fluorescen, la fluorescencia de estas paredes se atribuye a lignina, pero esto no excluye la presencia de taninos.

No se observaron diferencias apreciables en el modelo adulto de la epidermis de microsporangios y macrosporangios de las especies estudiadas. Los caracteres sobresalientes de la pared adaxial del esporangio se resumen en la Tabla 2.

Ontogenia del modelo epidérmico. Se llevó a cabo por haberse observado que la pared adaxial de ciertos esporangios de la misma planta daba reacciones de color diferente con el TBO. Por esa razón, se estudiaron todos los esporofilos de plantas adultas completas para ver qué modificaciones tenían lugar en el curso del desarrollo de la pared esporangial. El estudio de la ontogenia del modelo se efectuó en *Isoetes setacea* (Fig. 15) e *I. histrix* (Fig. 16).

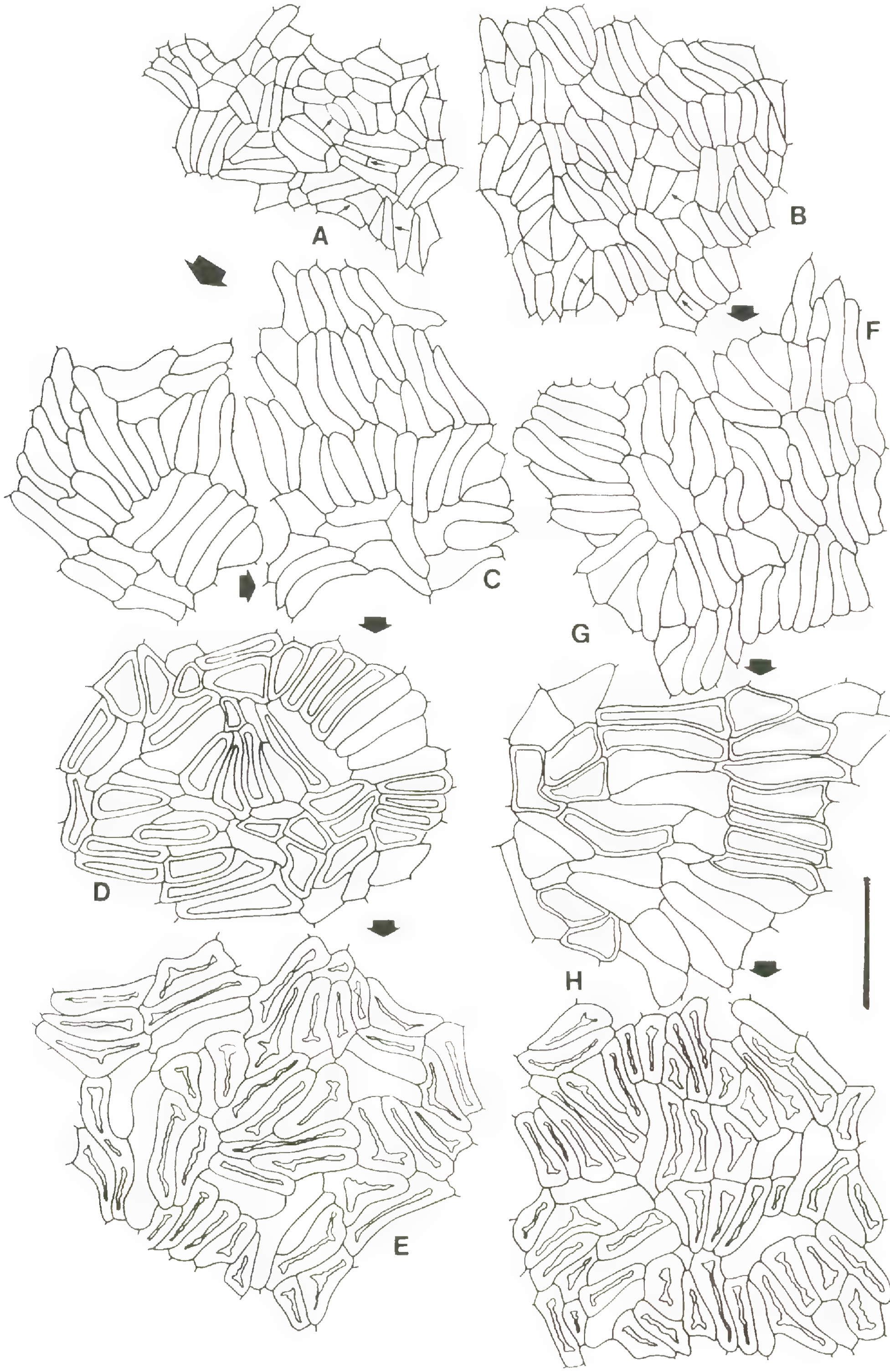

Figura 15. Ontogenia del modelo esporangial en *Isoetes setacea*. —A, B. Epidermis de macrosporangio y microsporangio juveniles más internos, con divisiones recientes y modelo tempranamente establecido. —C. Epidermis de macrosporangio en vías de maduración (contiene esporas inmaduras), con grupos celulares en parqué y células grandes con pared primaria. —D, E. Sucesivas etapas de maduración de la epidermis del macrosporangio; modelo en parqué, células con pared primaria cutinizada y células con pared secundaria lignificada. —F. Epidermis de microsporangio juvenil, modelo en parqué, células con pared primaria. G, H. Epidermis en sucesivas etapas de maduración. —G. Grupos celulares con pared secundaria engrosada no lignificada (microsporangio con esporas inmaduras). —H. Epidermis adulta del microsporangio. Flechas finas, divisiones recientes. Escala: 100 µm.

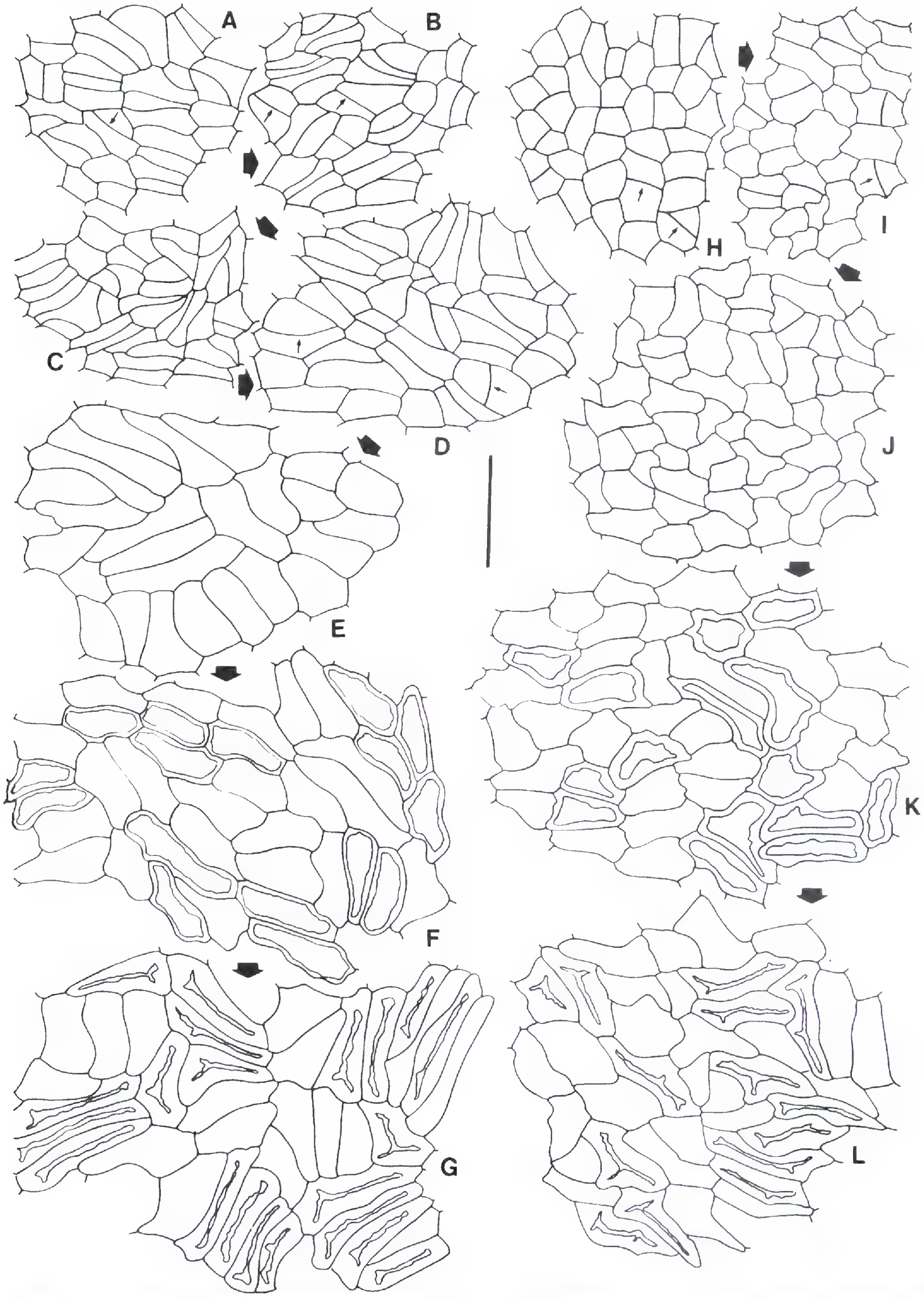

Figura 16. Ontogenia del modelo esporangial en *Isoetes histrix*. —A–D. Epidermis de macrosporangio juvenil más interno con divisiones recientes y modelo establecido tempranamente. —E. Epidermis de macrosporangio en vías de maduración (contiene esporas inmaduras), con grupos celulares en parqué y células grandes con pared primaria. —F, G. Sucesivas etapas de maduración de la epidermis del macrosporangio; modelo en parqué, células con pared primaria cutinizada y células con pared secundaria lignificada. —H, I. Epidermis de microsporangio juvenil, células con pared primaria débilmente cutinizada dispuestas con modelo poligonal. —J. Epidermis de microsporangio juvenil, células con pared primaria débilmente cutinizada dispuestas con modelo subsinuoso. K, L. Epidermis en sucesivas etapas de maduración. —K. Grupos celulares con pared secundaria engrosada no lignificada (microsporangio con esporas inmaduras). —L. Epidermis adulta del microsporangio. Flechas finas, divisiones recientes. Escala: 100 µm.

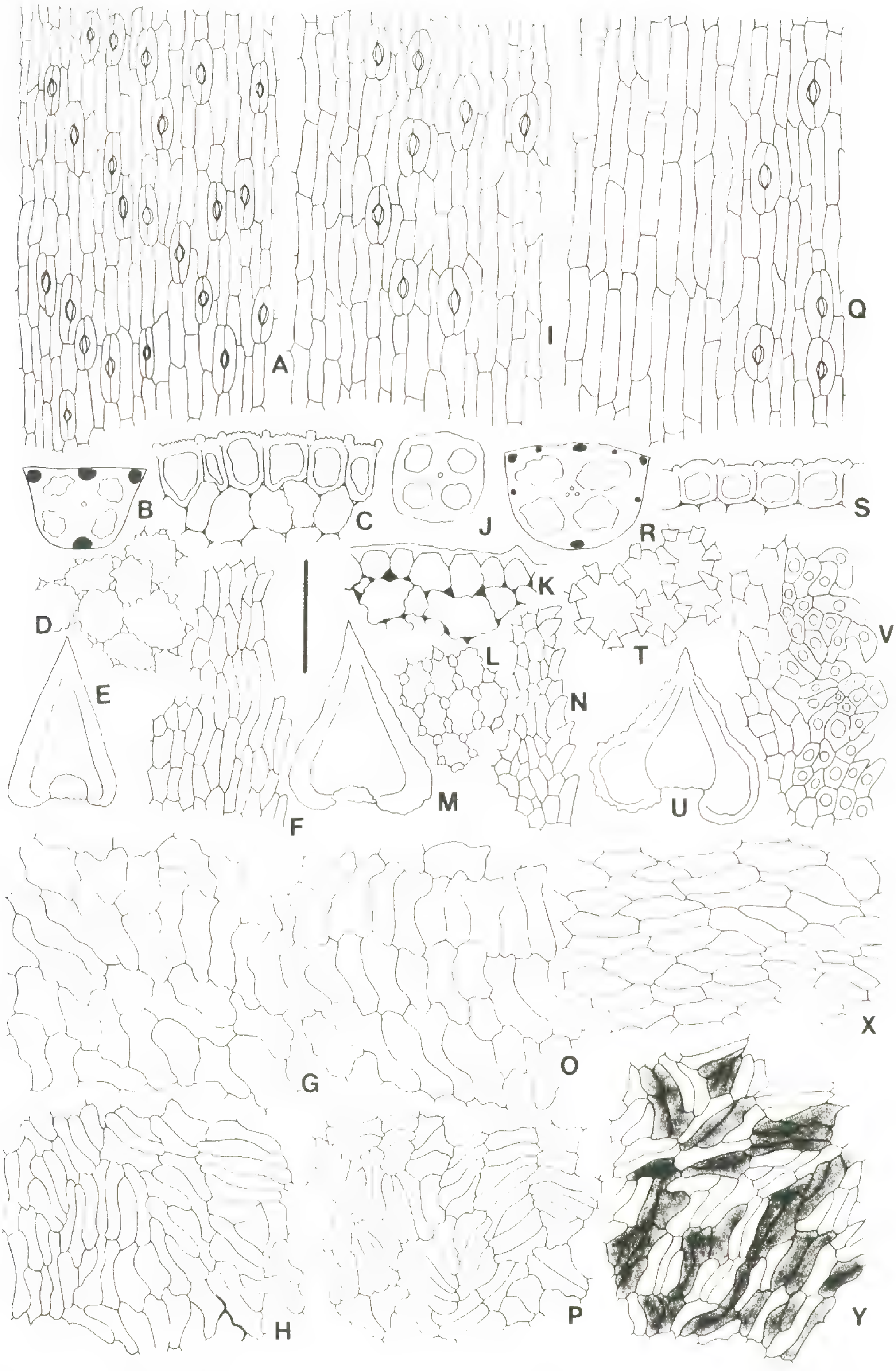

Figura 17. Caracteres foliares en especies de *Isoetes*. —A–H. *I. nuttallii*. —I–P. *I. orcuttii*. —Q–Y. *I. howellii*. A, I, Q. Modelos epidérmicos de los microfilos. B, J, R. Secciones foliares, en B y R, en negro, haces de colénquima. C, K, S. Epidermis de los microfilos en corte transversal, en C y S, con cuñas cuticulares sobresalientes, en K, en negro, colénquima hipodérmico lacunar. D, L, T. Diafragmas foliares. E, M, U. Lígulas. F, N, V. Epidermis del margen de las lígulas, en V, las

En *Isoetes setacea* el modelo epidérmico de los macrosporangios y microsporangios más jóvenes es similar (Fig. 15A, B). En ambos casos la epidermis juvenil muestra etapas iniciales semejantes y sus células tienen paredes primarias celulósicas muy débilmente cutinizadas o sin cutina (no se colorean con TBO). Son poligonales, más largas que anchas y tienen una disposición en grupos algo ordenados que, a la vez que aumentan de tamaño, definen el modelo en parqué típico de la mayoría de las especies descritas (Fig. 15C, F). En una etapa subsiguiente de maduración, comienza el engrosamiento de las paredes de algunos grupos celulares, que responden positivamente a la prueba de lignina con el TBO pero aún no se observa fluorescencia (Fig. 15D, G). La etapa adulta de la epidermis se caracteriza por la presencia de grupos de células con lumen muy escaso y paredes lignificadas que dan fluorescencia, alternando con grupos reducidos de células con paredes no lignificadas (Fig. 15E, H).

En *Isoetes histrix* (Fig. 16) la ontogenia de la epidermis esporangial es similar, pero difieren las etapas juveniles del modelo de macrosporangios y microsporangios. En el caso del macrosporangio (Fig. 16A–G), la ontogenia sigue el modelo tipo *I. setacea*, mientras que las etapas juveniles del microsporangio (Fig. 16H–L) se inician con un modelo poligonal de células isodiamétricas (Fig. 16H) que pasa por etapas de modelo subsinuoso (Fig. 16I, J) previas al establecimiento del tipo adulto, en parqué (Fig. 16L). Las etapas inmaduras de la pared de macrosporangios y microsporangios en las que se presentan grupos de células con paredes primarias y células con paredes secundarias son morfológicamente muy similares (Fig. 16F, K). Las microsporas y macrosporas están maduras en la etapa ilustrada en Figura 16G y 16L; hay microsporas ya diferenciadas pero sin la ornamentación característica en la etapa ilustrada en Figura 16J, y macrosporas ya diferenciadas pero no de tamaño adulto en la etapa ilustrada en la Figura 16F. Es decir, no hay sincronía en la maduración de la ornamentación esporal ni coincidencia en las etapas iniciales de la ontogenia en los esporangios de *I. histrix*.

CONSIDERACIONES SOBRE ALGUNOS GRUPOS DE ESPECIES

El caso de *Isoetes nuttallii* (Fig. 17A–H), *I. orcuttii* (Fig. 17I–P) e *I. howellii* (Fig. 17Q–Y) suministra un interesante ejemplo de aplicación de los caracteres aquí estudiados. Se trata de especies fenotípicamente muy similares, que conviven en charcas temporales. Las especies difieren en el modelo epidérmico, las relaciones l/a celulares, la densidad y dimensiones de los estomas, las ornamentaciones cuticulares, la presencia o ausencia de cuñas, las secciones foliares, la presencia y tipo de colénquima, los tipos de células de los diafragmas, la presencia y tipos de PIP, el contorno de la lígula, la duración de la función secretora marginal, el modelo epidérmico del velo y de los esporangios. *Isoetes howellii* es muy característica y se distingue fácilmente de las otras dos especies por la extensión del velo y la pigmentación del esporangio, pero para observar estos caracteres, las plantas deben estar maduras. *Isoetes orcuttii* e *I. nuttallii* se distinguen fundamentalmente por el tamaño de las hojas y de las macrosporas (Taylor et al., 1993). Un simple corte transversal de los microfilos, no necesariamente maduros, permite reconocer las tres especies por los caracteres de su anatomía foliar.

Este conjunto de caracteres es útil en otros grupos de especies, como *Isoetes brochonii*, *I. echinospora* e *I. lacustris*. La coincidencia de algunos caracteres morfológicos se debe a la relación de parentesco existente entre ellas (Taylor & Hickey, 1992). Las plantas de *I. lacustris* son muy semejante a las de *I. brochonii*, pero un examen de ambas especies muestra que *I. lacustris* se caracteriza por las estrías cuticulares gruesas y discontinuas, las cuñas cuticulares sobrelevadas, la lígula largamente secretora y la pared esporangial con un modelo en parqué cuyas células contactan mediante paredes curvas, mientras que *I. brochonii* tiene estrías irregulares y carece de cuñas cuticulares, su lígula se esclerosa tempranamente y la pared adaxial del esporangio tiene un modelo en parqué cuyas células contactan mediante paredes angulosas, rasgo éste que comparte con *I. echinospora*.

En el grupo de *Isoetes velata* se reúnen especies con esporas muy similares (macrosporas pustuladas con bulas de diferentes tamaños y microsporas equinadas o lisas a oscuramente papilosas). *Isoetes velata* subsp. *velata* (incluyendo *I. tenuissima* Boreau) se caracteriza por la presencia de velo casi completo; *I. adspersa* se segrega por la menor extensión del velo; *I. boryana* por la ausencia de bases foliares papiráceas persistentes y por microsporas lisas a sub-papilosas; *I.*

←
células con núcleos ilustrados indican área secretora. G, O, X. Modelos epidérmicos del velo. H, P, Y. Modelo epidérmico adaxial de los esporangios, en Y, las áreas oscuras indican células epidérmicas no lignificadas, conteniendo taninos. Escala: 100 µm en A, C, I, K, Q, S; 70 µm en D, L, T; 125 µm G, H, O, P, X, Y; 150 µm en F, N, V; 2 mm en B, J, R; 1.5 mm en E, M, U.

longissima, por la longitud de sus microfilos e *I. velata* subsp. *asturicensis*, por sus macrosporas casi lisas (Maire, 1952; Prada, 1986; Jermy & Akeroyd, 1993). Los caracteres que se utilizan para diferenciar estas especies pueden ser tan variables que en la práctica resulta difícil asignar un ejemplar a un nombre. Los caracteres analizados aquí indican que todas comparten las cutículas estriadas y excluyendo a *I. boryana*, comparten los estomas actinocíticos y las PIP verrucosas. *Isoetes adspersa* podría ser considerada una buena especie, que se distingue por la cutícula uniformemente estriada, las relaciones l/a celulares 1:1, el modelo epidérmico del velo subsinuoso, con células grandes y la pared esporangial con células tánicas, veteada externamente. Estos caracteres también podrían indicar que *I. boryana* es una buena especie caracterizada por la cutícula finamente estriada en toda la pared externa, el modelo epidérmico de los microfilos con relación l/a celular 1:1, el contorno de los estomas y el modelo poligonal de la epidermis del velo. *Isoetes longissima*, *I. velata* subsp. *velata* e *I. velata* subsp. *asturicensis* coinciden en casi todos los rasgos de los microfilos, de la lígula, del velo y del esporangio. *Isoetes velata* subsp. *velata* e *I. velata* subsp. *asturicensis* difieren únicamente en la cantidad de hacecillos de colénquima, más abundantes y con células más engrosadas en la subespecie tipo. De acuerdo con lo observado aquí, la reducción del colénquima en *I. velata* subsp. *asturicensis* se relacionaría con la altura y esta subespecie podría ser considerada tan sólo como una forma de *I. velata*, pero no un taxón diferente. *Isoetes longissima* tiene verrugas pécticas más esparcidas, con la superficie rugosa y células de los diafragmas con micro-pip (Prada & Rolleri, 2005), mayor tamaño de lígula, células epidérmicas, estomas y esporas. Estos caracteres, incluyendo su mayor ploidía, justificarían su reconocimiento como un taxón diferente, probablemente con nivel subespecífico.

Discusión

En las diagnosis de especies de *Isoetes* se usaron casi siempre caracteres cuantitativos como el número de lóbulos del cormo, la longitud y anchura de las hojas, el número de microfilos de las plantas, la anchura y extensión del ala membranosa marginal foliar, la extensión del velo, los tamaños esporales, etc., o bien caracteres cualitativos de difícil generalización, como el color de las hojas, la forma de la lígula y de los esporangios, las ornamentaciones esporales, etc. Las claves basadas en este tipo de caracteres no siempre permiten determinar con seguridad las especies.

Los caracteres foliares aquí estudiados tienen un valor diagnóstico variable. La presencia de una lígula secretora de mucílago y de una endodermis en los canales intrastelares y en las cámaras aeríferas de los microfilos son comunes a todas las especies y son caracteres genéricos en *Isoetes*. Los contornos de las secciones foliares, los tipos de colénquima (en haces, lacunar o parcialmente laminar), los tipos de células de los diafragmas foliares, la presencia o ausencia de PIP, la extensión de la endodermis en las cámaras aeríferas, la persistencia de la función secretora de la lígula o el esclerosamiento temprano de este órgano, y el grado de desarrollo de un labio amiláceo, son rasgos que caracterizan grupos de especies. La presencia y tipos de ornamentaciones cuticulares, las cuñas cuticulares sobresalientes, los márgenes papilosos, los modelos y las relaciones l/a celulares de las células epidérmicas de los microfilos, los tipos o combinaciones de tipos de PIP, los modelos y las relaciones l/a celulares de las células epidérmicas del velo, el modelo epidérmico de la pared adaxial de los esporangios y la naturaleza de las paredes celulares en estas epidermis, son caracteres específicos.

Aunque individualmente o de manera aislada se hayan estudiado algunos caracteres específicos de los mencionados arriba, tal y como se han presentado aquí no han sido utilizados previamente en el género. Los caracteres que parecen tener mayor valor diagnóstico son aquéllos relacionados con la epidermis de los microfilos. En el caso de las ornamentaciones y cuñas cuticulares, cuando aparecen, se diferencian tempranamente y son visibles en plántulas y plantas adultas (Prada & Rolleri, 2003). Si la epidermis es lisa, estriada o verrucosa, y si tiene o no cuñas sobresalientes, el carácter será apreciable en microfilos juveniles y en adultos, y será un rasgo característico de la especie.

Los caracteres estomáticos no son específicos. En muchas especies las células periestomáticas se disponen de forma aproximadamente actinocítica y en otras este ordenamiento no se observa (Prada & Rolleri, 2003). Cuando aparece la disposición actinocítica, las especies que la presentan también son afines por otros caracteres morfológicos. Las dimensiones de los estomas varían con un rango similar en especies de áreas geográficas diferentes.

La mayoría de las especies estudiadas de *Isoetes* tienen algún tipo de tejido mecánico colenquimático. La organización en haces no es el único tipo de colénquima en *Isoetes* aunque hasta ahora parece ser el más común (Prada, 1979a; Prada & Rolleri, 2003). Algunas especies desarrollan los tipos aquí descritos como lacunar y parcialmente laminar, e incluso puede presentarse una combinación de dos tipos de colénquima en la misma especie. La mayor diversidad

de tipos de colénquima se ha encontrado en especies de los páramos, pero también hay especies de tierras bajas, como *I. melanopoda* e *I. piedmontana*, en las que se presenta a la vez colénquima en haces y lacunar.

Pfeiffer (1922) señaló que la presencia de colénquima estaría influida por el hábitat y que tiende a estar ausente en las especies acuáticas. Pastore (1936) consideró que su presencia es un rasgo de madurez de las plantas, no condicionado por el hábitat. Prada y Rolleri (2003) señalaron que había una disminución de los haces en algunas especies acuáticas, pero sugirieron que en ciertos taxones podría ser un rasgo específico, ya que no todos los acuáticos estudiados carecían de colénquima. El hecho de que las plantas juveniles y adultas de *Isoetes lechleri* presenten colénquima lacunar y que en *I. velata* el colénquima en haces sólo esté bien desarrollado en plantas adultas podría indicar que todo lo relacionado con el colénquima tiene variación específica y representa una fuente de caracteres diagnósticos no desechable. La formación de colénquima como tejido de sostén es típica de *Isoetes* pero su relación con el hábitat, el grado de inmersión de las hojas u otros factores está aún sin probar.

Las PIP y los diafragmas foliares también presentan caracteres diagnósticos de interés. Las lobulaciones de las células de los diafragmas varían, pero aún no se conoce este carácter en un número suficiente de especies para valorar su utilidad. Hay especies muy similares, como *Isoetes nuttallii* e *I. orcuttii*, en las que el tamaño y forma de las células de los diafragmas varían, mientras que en especies diferentes y fácilmente distinguibles, como *I. anatolica* e *I. malinverniana*, las células de los diafragmas son muy similares. Las células globosas de los diafragmas no suelen presentar PIP y las lobuladas o estrelladas generalmente las presentan. Las PIP parecen más bien difundidas en *Isoetes*, y hasta ahora, los tipos o combinaciones de tipos son específicos (Prada & Rolleri, 2005).

Los canales intrastelares fueron señalados como de posible valor sistemático por autores recientes (Hall, 1971; Prada, 1979a; Takamiya et al., 1997). Aquí se ha observado que el número de los mismos es estable en la mayoría de las especies, con excepción de *Isoetes histrix*, donde aparece un número variable entre 1 y 5, aunque es posible que, por tratarse de una especie terrestre sometida a inundación estacional, estas variaciones tengan alguna relación con el hábitat. Hall (1971) sugirió que los canales llevan agua y no aire. Esta es una interesante afirmación en relación con lo anterior, ya que el mayor número de canales intrastelares en plantas anfibias o terrestres podría relacionarse con la necesidad obvia en éstas de evitar que los tejidos vasculares se inunden periódicamente, una función a la que contribuye la presencia de endodermis en los canales (Rolleri & Prada, 2004).

Se han encontrado dos tipos morfológicos principales de lígulas, las que tienen contornos ovados y las que tienen contornos lanceolados, con ligeras variaciones en la forma general y el tamaño en plantas de una misma especie o aún en microfilos de una misma planta. La lígula es un órgano secretor de mucílago, pero la extensión del área marginal secretora y la duración de esta función parecen ser específicas. No se ha podido establecer una relación entre el hábitat y estos caracteres morfológicos y fisiológicos de las lígulas, ya que el mismo tipo se presenta en especies de diferentes hábitats (lígulas de *Isoetes boryana, I. durieui, I. histrix, I. howellii, I. lacustris, I. setacea, I. velata* susbp. *velata, I. velata* susbp. *asturicensis*). Especies que conviven pueden presentar lígulas distintas, como es el caso de *I. nuttallii* e *I. orcuttii* en relación con *I. howellii*. Se destacan por su temprana lignificación y el cese de la función secretora las lígulas de especies totalmente distintas como son *I. andina, I. brochonii* e *I. killipii*. El estudio de un número más amplio de taxones podría indicar eventuales líneas de especialización de este órgano en el conjunto del género, pero el grado de accesibilidad del carácter, el hecho de que en muchos casos se trata de una estructura efímera que se pudre con facilidad o que se destruye si se manipula material seco sin una restauración adecuada, hace que la evaluación de este rasgo sea algo más difícil que en el caso de otros caracteres foliares.

En la mayoría de las especies estudiadas aquí, el labio varía más que la lígula en los microfilos de la misma especie. Tanto el velo como el labio son estructuras que contienen almidón y podrían tener función similar. Goswami (1976) señala que el labio tiende a reducirse en las especies que presentan velo. Según ese autor, en el curso de la ontogenia, el labio o la lígula podrían ser estructuras caducas y el labio sería más importante que la lígula en especies sin velo. La relación entre el desarrollo del labio y el del velo no es tan clara: *Isoetes anatolica* carece de velo y tiene un labio generalmente bien desarrollado; *I. setacea*, sin velo, e *I. histrix*, con velo, tienen labios similares, cortos y reducidos, e *I. melanopoda*, con velo parcial, tiene un labio más desarrollado que el de *I. histrix*, con velo completo y similar al de *I. anatolica*, que carece de velo.

La epidermis del velo varía específicamente. En las especies estudiadas predominan los modelos poligonales, pero varían las relaciones l/a y los tamaños celulares. Es posible que los tamaños, como en otras epidermis (Barrington et al., 1986), tengan relación con la ploidía interna, pero la variación observada es

específica. En combinación con otros rasgos foliares permite caracterizar las especies.

El modelo epidérmico del esporangio difiere del modelo de los microfilos y del modelo del velo y tanto el patrón celular adaxial como el color externo del esporangio son caracteres de variación interesante. En las especies estudiadas y en otros trabajos en los que se ilustra la epidermis adaxial del esporangio, el modelo más frecuente es el que aquí se ha llamado en parqué, formado por células con pared primaria y sin taninos. Este es también el modelo básico de los esporangios en los que se combinan grupos de células con pared primaria y otros con paredes lignificadas o con taninos. En todos los modelos restantes (poligonales, subsinuosos o sinuosos) las células tienen paredes primarias y no contienen taninos.

En las especies estudiadas el modelo epidérmico de microsporangios y macrosporangios adultos coincide. Las etapas juveniles de esos modelos no necesariamente coinciden en uno u otro tipo de esporangio. No es raro que las plantas de *Isoetes* tengan macrosporangios maduros cuando los microsporangios están aún en etapas juveniles del desarrollo de su pared, algo que es necesario tener en cuenta al utilizar el rasgo para caracterizar a una determinada especie. Sólo los esporangios con pared adulta tienen esporas con su ornamentación completamente desarrollada, lo que podría explicar los casos de algunas de las especies cuyas microsporas se han descrito como de ornamentación variable cuando en realidad podría tratarse de diferentes etapas de maduración de la pared del esporangio y de las esporas que contiene.

Varios autores utilizaron el color externo visible de la pared esporangial como carácter diagnóstico y en todos los casos los tipos de pared esporangial que mencionan podrían clasificarse en los que se han observado aquí. Maire (1952) lo utilizó para distinguir entre taxones del grupo de *Isoetes velata*, y observó paredes veteadas en *I. adspersa*, en las que el color externo se debe a taninos. Hall (1971) ilustró diferencias en la epidermis esporangial de *I. abyssinica* Chiovenda, *I. nigritiana* A. Braun e *I. tenuifolia* A. C. Jermy. Ilustró un modelo similar al de *I. adspersa*, en *I. abyssinica* y un modelo en parqué con células con paredes primarias alternando con otras con paredes engrosadas, en *I. tenuifolia*. El esporangio de *I. nigritiana* no está ilustrado pero lo describe como similar al de *I. tenuifolia*, con paredes celulares menos engrosadas. Hall (1971) sugiere que esas paredes engrosadas no estarían lignificadas, por su reacción negativa ante floroglucina clorhídrica, pero esta prueba no es adecuada para lignina de pteridófitos (Johansen, 1940).

Kott y Britton (1985) citaron esporangios con paredes coloreadas y no coloreadas en especies norteamericanas de *Isoetes*. La ilustración que presentan de *I. riparia* Engelm. sugiere un modelo en parqué del tipo formado por células con paredes primarias y células con paredes lignificadas, pero esos autores no analizaron el origen del color e indicaron que la coloración externa estaba correlacionada con otros rasgos, como los esporales. Hickey (1986) incluyó la pigmentación esporangial en un análisis cladístico y consideró que el color sería un carácter primitivo. En ese trabajo no se distingue tampoco el origen del color, aunque se sugiere que los caracteres con los que se asocia (filopodios, color de las escamas, etc.) estarían intimamente relacionados.

En las especies estudiadas aquí, los esporangios pueden ser externamente hialinos, veteados de castaño o uniformemente castaños. El origen del color es variable. Las paredes externamente veteadas siempre se han visto en el caso de modelos que presentan células taníferas, la asociación entre estas células y esporangios de pared veteada podría ser el caso más frecuente. Las paredes externamente sin color se encuentran tanto en modelos con paredes primarias no lignificadas como en modelos con paredes engrosadas y lignificadas. Por ejemplo, los esporangios de *Isoetes andina* e *I. piedmontana* son externamente coloreados y tienen células lignificadas, mientras que en *I. histrix* e *I. setacea*, también hay células lignificadas pero no color externo. En la bibliografía sobre *Isoetes* se le ha conferido valor diagnóstico al color de la pared del esporangio. Aquí se amplían las posibilidades de utilización sistemática del carácter, agregando el modelo epidérmico, el origen del color y la naturaleza de sus paredes.

Hay toda una etapa del estudio botánico que parece haberse omitido en *Isoetes*. Superado el tratamiento tipológico y el análisis de caracteres morfológicos externos, el género ha sido enfocado desde un modelo llamado biológico, que no dependería de límites morfológicos sino reproductivos (Hickey et al., 1989). Ese enfoque ha pasado por alto la consideración de algunos caracteres morfológicos más estables que los externos, no dependientes de variables como edad de las plantas, fenología y condiciones de crecimiento. El número de especies de *Isoetes* estudiadas en detalle es bajo en relación con el número de especies descritas para el género. Un mejor conocimiento de los caracteres de cada especie permitiría establecer un ordenamiento más natural y sería más fácil ver las eventuales líneas morfológicas o combinaciones de rasgos en el género como un todo y analizar el significado de cada una de ellas. Las propuestas de división infragenérica y de interpretación de la filogenia del género basada en los caracteres morfológicos externos tradicionales (Hickey, 1986;

Taylor & Hickey, 1992) o esporales (Pfeiffer, 1922) no se han visto avaladas por los resultados de otro análisis filogenético basado en el estudio de las secuencias del ADN plastidial (Ryding & Wikström, 2002).

Con frecuencia se afirma que *Isoetes* es un género con escasos caracteres de valor diagnóstico. Sin embargo, de acuerdo con nuestras observaciones, las especies presentan grupos de caracteres independientes de la ornamentación de sus esporas, que resultan útiles y que deberían ser incluidos en las descripciones. Las plantas de *Isoetes* pueden ser externamente muy semejantes, pero caracteres como los que se han estudiado aquí, cuya apreciación no es difícil ni engorrosa, permitirían determinarlas con mayor seguridad y se dispondría de un mayor número de rasgos estables de aplicación en estudios cladísticos o de relaciones filogenéticas en el género.

El conjunto de caracteres analizados tiene un valor diagnóstico interesante: complementa los esporales frecuentemente utilizados como básicos en el género; puede reemplazar a los esporales en ciertos casos; permite el análisis de muchas colecciones en las que las esporas no están presentes; permite distinguir entre especies diferentes pero fenotípicamente similares; permite determinar ciertas especies en distintas etapas fenológicas y reconocer esas etapas. Son caracteres que están siempre disponibles y se requieren pequeñas cantidades de material (de herbario o fresco) para su estudio.

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Apéndice 1. Especímenes Representativos de las Especies de *Isoetes* Estudiadas Anatomicamente en Este Trabajo.

Isoetes adspersa A. Braun. ARGELIA. **Orán**: Mare du Djebel Santo, 11 Mayo 1883, *Debeaual s.n.* (MA); Rou Sfer, Foret de M'Sila, 26 Abr. 1891, *Doumergue s.n.* (MA).

Isoetes anatolica Prada & Rolleri, Bot. J. Linn. Soc. 147 (2): 215. 2005. TURQUÍA. **Bolu**: S de Abant Golu, *Aedo et al. 6181* (MA), *6185* (MA).

Isoetes andina Spruce ex Hook. f. COLOMBIA. **Cundinamarca**: Laguna de Chisacá, *Icosta-Arteaga 250* (MA); Macizo de Sumapaz, Andabobos, *Cuatrecasas 27038* (MA). ECUADOR. **Chimborazo**: 10 km NE of Alao at Cuspipacha, 6 Mayo 1982, *Øllgaard et al. s.n.* (MA).

Isoetes boliviensis U. Weber. BOLIVIA. **La Paz**: Entre Escoma y Hualcapayo, *Ceballos et al. Bo-672* (MA).

Isoetes boryana Durieu. FRANCIA. **Gironde**: Etang de Cazaux, 7 Jun. 1897, *Giraudias s.n.* (MA); Sep. 1902, *Casares Gil s.n.* (MA).

Isoetes brochonii Motelay, Extr. Actes Soc. Linn. Bordeaux, 5 série, 45: 45. 1892. ESPAÑA. **Lérida**: Estany de la Pera, *Carrasco 19902 & Martín Blanco* (MACB). FRANCIA. **Ariège**: vallée d'Orlu, près Aix-les-Thermes, lac de Naguilles, *Veyraut et al. s.n.* (MA). **Pyrénées Orientales**: Les Bouillouses, Lac du Vive, 20 Ago. 1989, *Prada s.n.* (MACB); Etang des Dougnes, Sep. 1994, *Herrero s.n.* (MACB).

Isoetes durieui Bory de Saint-Vincent, Compt. Rend. Hebd. Séances Acad. Sci. 18: 1166. 1844. ESPAÑA. **Cádiz**: Ubrique, Collado del Cuervo, *Silvestre TF 75* (SEV); Entre Tarifa y Algeciras, *Luque et al. 2176/78* (SEV). **Madrid**: entre Hoyo de Manzanares y Colmenar Viejo, Jun. 1988, *Prada s.n.* (MACB); Torrelodones, Mayo 2001, *Prada s.n.* (MACB).

Isoetes echinospora Durieu. ANDORRA. Estanys de Pessons, 9 Ago. 1977, *Prada s.n.* (MACB). ESPAÑA. **Soria**: Laguna Larga de Urbión, 23 Ago. 1972, *Montserrat s.n.* (JACA); Laguna Negra de Urbión, 21 Sep. 1978, *Rivas Martínez s.n.* (MACB). FRANCIA. **Pyrénées Orientales**: Les Bouillouses, Lac du Vive, 20 Ago. 1989, *Prada s.n.* (MACB); Lac Noir, Sep. 1994, *Herrero s.n.* (MACB); Lac de Pradeillles, 8 Sep. 1988, *Prada s.n.* (MACB).

Isoetes engelmanii A. Braun. ESTADOS UNIDOS DE AMÉRICA. **Massachusetts**: Hampshire Co., Haydenville, Northampton Reservoir, *Ahles 77676* (MA).

Isoetes histrix Bory de Saint-Vincent, Compt. Rend. Hebd. Séances Acad. Sci. 18: 1167. 1844. ESPAÑA. **Cáceres**: Pasado Plasencia, 9 Abr. 1978, *Prada s.n.* (MACB); Abadía, hacia el Embalse de Gabriel y Galán, 30 Mayo 1985, *Ramos et al. s.n.* (MACB). **Cádiz**: entre Tarifa y Algeciras, *Luque et al. 2176/78* (SEV); Sierra del Aljibe, Puerto de Gáliz, *Silvestre TF 61* (SEV). **Ciudad Real**: Alcoba, Parque Nac. de Cabañeros, 1 Jun. 2000, *Bernal & Hernández s.n.* (MACB). **Coruña**: Torre de Hércules, 10 Oct. 1976, *Prada s.n.* (MACB). **Huelva**: Laguna de la Dehesilla, 22 Feb. 2002, *Fuertes s.n.* (MACB); Coto de Doñana, Abr. 1979, *Cubas s.n.* (MACB). **Madrid**: Torrelodones, Mayo 2001, *Prada s.n.* (MACB). **Pontevedra**: Cabo Silleiro, 16 Feb. 1989, *Prada s.n.* (MACB). FRANCIA. **Finisterre**: Bigouden, 23 Abr. 2002, *Citoleux s.n.* (MACB). PORTUGAL. **Algarve**: alrededores de Faro, Pinhal do Ludo, 25 Abr. 1951, *Sobrinho & Mendes s.n.* (COI). **Beira Litoral**: Leiria versus Marinha Grande, 8 Jun. 1956, *Pinto da Silva et al. s.n.* (LISE). MARRUECOS. **Rabat**: Temara, 10 km S de Rabat, 20 Feb. 1978, *Damblon 78/2* (MA).

Isoetes howellii Engelmann, Trans. Acad. Sci. St. Louis 4: 385. 1882. ESTADOS UNIDOS DE AMÉRICA. **California**: Sacramento Co., Howard Ranch, 25 Abr. 2001, *Molina s.n.* (MACB).

Isoetes killipii C. V. Morton. COLOMBIA. **Boyacá-Santander**: Charalá, Páramo de la Rusia, *Fernández Alonso et al. 6104* (MA).

Isoetes lacustris L. ANDORRA. Estanys Furcat, 13 Ago. 1949, *Losa & Montserrat s.n.* (MAF). BULGARIA. Montes Pirin, Lago Muratovo, *Burgaz et al. s.n.* (MACB). ESPAÑA. **Lérida**: Estanys d'Unarre (Pallars Sobirà), 27 Jun. 1985, *Masalles & Ninot s.n.* (MACB); Est. de la Pera, 19 Ago. 1989, *Prada s.n.* (MACB); Lac d'Anglade, Vieille Aure, 12 Ago. 1971, *Montserrat s.n.* (JACA); Est. hacia el Est. Negre, 6 Ago. 1958, *Montserrat s.n.* (JACA); Est. Cavallers, pr. Caldes de Bohí, 22 Jul. 1944, *sine coll.* (MA). FRANCIA. **Ariège**: Aix-les-Thermes, lac de Naguilles, *sine coll., s.n.* (MA). **Pyrénées Orientales**: Les Bouillouses, Lac Long, 7 Ago. 1977, *Pardo & Prada s.n.* (MACB); Lac d'Oredon, *Dussaussois FR-65* (MACB). ESTADOS UNIDOS DE AMÉRICA. **Wisconsin**: Oneida Co., Pelican Lake, Mayo 1989, *Taylor & Prada s.n.* (MACB).

Isoetes lechleri Mett. BOLIVIA. **La Paz**: Omasuyos, entre Achacachi y Sorata, cerca de Humanata, *Ceballos et al. BO-580* (MA); Omasuyos, pr. Achacachi, *Fernández Casas 6619* (MA).

Isoetes longissima Bory de Saint-Vincent, Compt. Rend. Hebd. Séances Acad. Sci. 18: 1165. 1844. ESPAÑA. **Coruña**: Teijeiro, Río Mandeo, 10 Oct. 1976, *Prada s.n.* (MACB). **Lugo:** Vilalva, Codesido, Río San Martiño, 5 Sep. 1992, *Soñora s.n.* (MA).

Isoetes malinverniana Cesati & De Notaris, Ind. Sem. Horti Genuensis 1858: 36. 1858. ITALIA. **Alejandría**: pr. Isarno et Vignale, 1910, *Gola s.n.* (MA). **Vercelli**: Oldenico, *Raynal 20885* (MA).

Isoetes melanopoda J. Gay & Durieu. ESTADOS UNIDOS DE AMÉRICA. **Georgia**: Butts Co. pr. Jackson, 10 Mayo 1989, *Luebke & Prada s.n.* (MACB).

Isoetes novo-granadensis H. P. Fuchs. COLOMBIA. **Cundinamarca**: de Villapinzón a Umbita, *Fernández et al. 15560* (MA).

Isoetes nuttallii A. Braun ex Engelm. ESTADOS UNIDOS DE AMÉRICA. **California**: Sacramento Co., Howard Ranch, 25 Abr. 2001, *Molina s.n.* (MACB).

Isoetes orcuttii A. A. Eaton. ESTADOS UNIDOS DE AMÉRICA. **California**: Sacramento Co., Howard Ranch, 25 Abr. 2001, *Molina s.n.* (MACB).

Isoetes piedmontana (N. Pfeiffer) C. F. Reed. ESTADOS UNIDOS DE AMÉRICA. **Georgia**: Lithonia, Roch Chapel Road, 10 Mayo 1989, *Luebke & Prada s.n.* (MACB).

Isoetes setacea Lam. ESPAÑA. **Cáceres**: pr. Mérida, 7 Abr. 2004, *Prada & Rolleri s.n.* (MACB); Río Ayuela, *Cubas & Prada s.n.* (MA). **Huelva**: Laguna de la Dehesilla, 22 Feb. 2002, *Fuertes s.n.* (MACB). **Madrid**: Hoyo de Manzanares, 29 Ago. 1988, *Prada s.n.* (MACB).

Isoetes storkii T. C. Palmer. COSTA RICA. **San José**: Dota Copey, Cerro Vueltas, *Castroviejo 15143SC & Sánchez* (MA).

Isoetes velata subsp. *velata* A. Braun. CÓRCEGA. **Suartone**, Tre Padule, 12 Jun. 1981, *Lambinon et al. s.n.* (MA). ESPAÑA. **Ávila**: Sierra de Gredos, Laguna Grande, *Fernández Casas 823 & García Guardia* (MA). **Cáceres**: Arroyo del Mejoral, 10 Jun. 1976, *Prada s.n.* (MACB). **Ciudad Real**: Valle de Alcudia, 22 Jun. 1983, *Prada s.n.* (MACB). **Huelva**: Coto de Doñana, Oct. 1976, *Castroviejo s.n.* (MACB). **Madrid**: Gargantilla del Lozoya, *Prada s.n.* (MACB). FRANCIA. **Indre**: Chateau Roux, Étang du Sault, 18 Jul. 1959, *Berger s.n.* (MA). **Var**: N de Fréjus, Bois de Palayson, *De Retz 65107* (MA). **Vienne**: St. Léomer, *Violleau s.n.* (MA). ARGELIA. Pr. Alger, Marais de la Rassanta, *Malato Beliz 631* (MA). MARRUECOS. Bab Taza, 26 Jun. 1930, *Font Quer s.n.* (MA). **Rabat**: Vallée du Regreg, pr. Souk-el-Arba-des-Lehoul, *Lhenuy & Sauvage 14402* (MA).

Isoetes velata subsp. *asturicensis* (Laínz) Rivas Mart. & Prada, Lazaroa 2: 327. ESPAÑA. **Asturias**: Leitariegos, laguna de Arvás, 30 Jul. 1976, *Pardo & Prada s.n.* (MACB); Turbera de Reconcos, próximo Gillón, Cangas del Narcea, *Aedo et al. CA3332b* (MA); Tchagueño d'Abaxo, próximo Cerredo, Degaña, *Aedo et al. CA3345* (MA). **Salamanca**: Sierra de Béjar, El Trampal, *Nieto 2746GN et al.* (MA).

FLORAL BIOLOGY OF NORTH AMERICAN *OENOTHERA* SECT. *LAVAUXIA* (ONAGRACEAE): ADVERTISEMENTS, REWARDS, AND EXTREME VARIATION IN FLORAL DEPTH[1,2]

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Abstract

We studied the floral biology of five North American members of *Oenothera* L. sect. *Lavauxia* (Spach) Endl. (Onagraceae L.) in field and common greenhouse settings. *Oenothera* sect. *Lavauxia* floral morphology ranges from small, cleistogamous flowers (*O. flava* subsp. *flava* (A. Nels.) Garrett in Garrett) to some of the longest-tubed flowers in North America (*O. flava* subsp. *taraxacoides* (Wooton & Standl.) W. L. Wagner). Our goal was to compare qualitative and quantitative aspects of floral advertisement and reward among taxa in section *Lavauxia*. All taxa are night-blooming and self-compatible, have yellow petals with ultraviolet reflectance, and produce floral scents dominated by nitrogenous compounds and monoterpenes. Methyl nicotinate is present in the fragrances of all taxa of section *Lavauxia* regardless of flower size or putative mating system. Because this floral volatile is largely absent from other *Oenothera* species, we hypothesize that it is a synapomorphy for section *Lavauxia*. The rare *O. acutissima* W. L. Wagner, which is endemic to the Uintah Mountains, is polymorphic for odors dominated by linalool- or ocimene-derived compounds. Field observations in its type locality in northeastern Utah, U.S.A., revealed frequent floral visitation by crepuscular hawkmoths during the first 1.5 hours after anthesis, a pattern common to *O. flava* subsp. *taraxacoides* and other large-flowered *Oenothera* throughout western North America. Quantitative aspects of floral advertisement (flower size, scent emission) and reward (nectar volume) are dramatically reduced in putatively autogamous taxa (*O. flava* subsp. *flava*, *O. triloba* Nutt.), whereas qualitative aspects (flower color, scent, and nectar chemistry) remain comparable. All taxa could be distinguished through ordination of characters related to flower size, herkogamy, and scent chemistry. Extreme nectar tube length variation across the range of *O. flava* renders this an excellent model system for measuring the costs and mechanisms of shifts between outcrossing and autogamy.

Key words: biogeography, floral scent, fragrance, nectar, night-blooming, *Oenothera*, Onagraceae, pollination.

The genus *Oenothera* L., with about 120 species native to the Americas, has long served as a model system for the study of evolutionary pattern and process in flowering plants (Raven, 1979, 1988, and references therein). The basic floral ground plan is fairly conserved across the genus with tetramerous flowers, white, pink, or yellow-colored petals, and a long, tubular hypanthium. However, there have been numerous evolutionary shifts between outcrossing and autogamy, nocturnal and diurnal anthesis, annual and perennial habit, and xeric and mesic habitat specialization in this genus. Recent efforts to establish well-supported phylogenetic hypotheses for this genus and its closest relatives in the Onagraceae L. (Levin et al., 2003a, 2004) have been motivated in part by the goal of understanding the frequency of these shifts and

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their underlying processes. In parallel, we and our colleagues have initiated studies of evolutionary transitions in reproductive biology and life history strategies in several monophyletic sections of *Oenothera*, including section *Anogra* (Spach) Engl. (Evans et al., 2005), section *Calylophus* (Spach) Torr. & A. Gray, section *Pachylophus* (Spach) Endl. (R. A. Raguso, in prep.), and in *Gaura* L. (Clinebell et al., 2004).

Here we examine the floral biology of the North American species of *Oenothera* sect. *Lavauxia* (Spach) Endl. The recognition of *Lavauxia* as a distinctive entity dates from early in the study of Onagraceae, either as a genus (Spach, 1835: 367; Raimann, 1893) or a subgenus of *Oenothera* (Endlicher, 1840: 1190; Jepson, 1923–1925: 680). As monographed by Munz (1930) and amended by Wagner (1981, 1986), *Oenothera* sect. *Lavauxia* presently consists of three yellow-flowered North American species, *O. acutissima* W. L. Wagner, *O. flava* (A. Nels.) Garrett in Garrett (including *O. flava* subsp. *flava* and *O. flava* subsp. *taraxacoides* (Wooton & Standl.) W. L. Wagner), and *O. triloba* Nutt., and two white-flowered South American species, *O. acaulis* Cav. and *O. centauriifolia* (Spach) Steud. *Oenothera triloba* is a small-flowered, self-compatible spring annual or biennial herb native to south-central U.S.A. This species is broadly parapatric with the western members of section *Lavauxia*, which are perennial herbs occurring throughout montane western North America into central Mexico (Wagner, 1986). Typical *O. flava* is a small-flowered, self-compatible plant with a very broad distribution, whereas *O. flava* subsp. *taraxacoides* is a large-flowered plant known only from disjunct, high-elevation populations in southwestern U.S.A. and northern Mexico. Wagner (1986) combined these two taxa as subspecies of *O. flava* on the grounds that small-flowered, autogamous individuals and large-flowered, modally outcrossing individuals represent extreme phenotypes of a single species that intergrade extensively in zones of contact. In contrast, Wagner (1981) segregated large-flowered, self-compatible but presumably outcrossing plants endemic to the mountains of northwestern Colorado and northeastern Utah, U.S.A., as a new species, *O. acutissima* (Wagner, 1981), citing several vegetative and reproductive autapomorphies that distinguish it from *O. flava*, with which it co-occurs. Subsequently, *O. acutissima* was treated as a variety of *O. flava* by Welsh (1986), but the poor viability (and sterility) of artificial hybrids between *O. acutissima* and *O. flava* (Wagner, 1981) compels us to recognize *O. acutissima* sensu Wagner (1986). Regardless of nomenclatural treatment, the western members of *Lavauxia* are remarkably variable in floral characters, from the short-tubed (less than 6 cm), often cleistogamous *O. flava* subsp. *flava* to the extremely long-tubed (up to 18 cm) *O. flava* subsp. *taraxacoides*, which are among the deepest flowers in the North American flora (Gregory, 1964; Grant, 1983; see Fig. 1).

We studied floral traits (i.e., color, morphology, scent, nectar) associated with pollinator attraction and reward in only the North American species of section *Lavauxia*, because the South American species (*Oenothera acaulis* and *O. centauriifolia*) were not available to us at the time of study. Given the biogeographic, morphological, and taxonomic patterns described above, we sought to address several questions with such data.

(1) Are floral scent and nectar (or components thereof) reduced or absent in the small-flowered, putatively autogamous flowers of *O. triloba* and *O. flava* subsp. *flava*? The dramatic difference in visual display between these and outcrossing taxa (*O. acutissima*, *O. flava* subsp. *taraxacoides*) suggests reduced selective pressure for floral advertisement and reward when self-pollination is the predominant strategy.

(2) Regarding components of floral fragrance, are plants of the two subspecies of *O. flava* more similar to each other than to plants of *O. triloba* and *O. acutissima* or does fragrance chemistry mirror pollination strategy, rather than phylogenetic affinity?

(3) Do individuals from two isolated populations of *O. flava* subsp. *taraxacoides* share a uniform phenotype or do floral traits vary distinctively between populations? The mountaintop distribution of these plants suggests that their floral traits may have diverged, either through genetic drift or local adaptation (Slentz et al., 1999; Boyd, 2002).

(4) Are flowers of the narrow endemic *O. acutissima* visited by the same spectrum of medium- to long-tongued hawkmoths that pollinate other *Oenothera* throughout western North America (e.g., Wagner et al., 1985)? Pollinator data are especially needed for this geographically restricted entity, which could be vulnerable to extinction due to habitat loss or modification.

The results of this study will provide a baseline for quantitative investigations of the costs, benefits, and evolution of floral advertisement and reward in this fascinating lineage.

Methods

Plant Care

Ten to 60 individuals of each taxon were cultivated in a greenhouse at the University of South Carolina, Columbia, South Carolina, where they were studied in

Figure 1. Floral variation in *Oenothera* sect. *Lavauxia*. —A. Newly opened flowers of *O. triloba*; note lack of strong herkogamy. —B. Nectar tube (hypanthium) length variation from putative autogamy (*O. triloba*, left, *O. flava* subsp. *flava*, center) to putative outcrossing (*O. flava* subsp. *taraxacoides*, right). —C. Side-by-side comparison of nectary depth between *O. flava* subsp. *taraxacoides* (right) and *Angraecum sesquipedale* Thouars (left), Darwin's Malagasy Star Orchid. —D. Differences in floral diameter between *O. flava* subsp. *taraxacoides* (left) and *O. flava* subsp. *flava* (right). —E. *Hyles lineata* leaving flower of *O. flava* subsp. *taraxacoides* in Sacramento Mtns., Otero Co., New Mexico. Photos in A–D by R. A. Raguso. Photo in E by M. S. Singer, with permission. All scale bars = 1 cm.

March–April 2002. *Oenothera acutissima* and *O. triloba* plants were transplanted from the field in July 2001 and March 2002, respectively. Plants from three populations of *O. flava* were grown from seed germinated in September 2001. Plants were grown in 60:40 potting soil:sand mix, bottom-watered daily, and fertilized with Miracle-Gro (15% N:30% P:15% K) once per month for the duration of the study. Voucher specimens were deposited at ARIZ, US, and USCH; collection numbers and source localities are provided in Appendix 1.

VISUAL REFLECTANCE

The spectral properties of newly opened flowers were measured using a Spectral Instruments (Tucson, Arizona) SI-440 CCD array UV-VIS Spectrophotometer, connected by a 400 µm fiber optic probe to a Labsphere (North Sutton, New Hampshire) 9 cm ID integration sphere. We used a 10 W tungsten light source to collect reflectance data from 350 (ultraviolet) to 700 nm (infrared) wavelengths from freshly excised flowers placed over a black felt cloth background. Spectral reflectance from upper (adaxial) petal surfaces was measured at both distal and proximal positions, as we anticipated a central "target pattern" of contrasting ultraviolet-absorbance and reflectance across the flower (Dement & Raven, 1974). Data were collected as percent reflectance relative to a white pigment (Duraflect) standard, with negligible (0.5%–1% of standard) signal contributed by the black cloth background.

NECTAR AND FLORAL MORPHOLOGY

Nectar volume was collected from at least 10 newly opened flowers of each species at dusk, using 5 or 10 µl glass capillary micropipettes. Because of the unusual length and narrowness of the floral tubes, nectar collection required floral dissection with a razor, but care was taken to avoid contaminating or diluting nectar with other plant fluids (Cruden et al., 1983). We measured sugar concentration in 5 µl aliquots from each sample with a hand-held refractometer (Leica, Brix50) designed to measure 0%–50% sucrose equivalents by weight, in units of 0.25%. Floral morphological measurements were taken on freshly excised flowers. We used a metric ruler to measure floral diameter (the greatest distance across the open corolla limbs, perpendicular to the nectar tube), tube flare (diameter across the mouth of the nectar tube, not including corolla limb), floral depth (length of nectar tube from mouth to ovary), stamen length and style length (distance from the ovary to the longest stamen and to the stigma, respectively), and herkogamy (difference in length between stigma and longest anther), to the nearest 0.5 mm. Fresh mass was recorded after nectar removal using a Mettler analytical balance, to the nearest 0.001 g. Flowers then were dried at 50°C for 24 hr., and dry mass was recorded on the same balance. Because plants of each species opened one to two flowers per night, most floral measures included flowers from different individuals.

FLORAL SCENT COLLECTION AND ANALYSIS

Floral odors were collected using two complementary methods. The first method, solid-phase microextraction (SPME) (Zhang & Pawliszyn, 1993), was combined with gas chromatography–mass spectrometry (GC-MS) to optimize the quality of mass spectra for scent compound identification. Headspace bags were prepared from Reynolds (nylon resin) oven bags using an impulse heat sealer, as described by Raguso et al. (2003a). Bags were placed over living, uncut flowers and cinched with plastic ties. Parallel collections were made from *Oenothera* leaves and greenhouse air in order to identify vegetative and ambient contaminants in floral samples. In other analyses, 5–10 µl samples of nectar were spotted onto filter paper wedges, sealed within headspace bags, and analyzed for nectar odors as described by Raguso (2004). All samples were equilibrated for 15 minutes, then allowed to adsorb onto a 100 µm polydimethylsiloxane (PDMS) SPME fiber for an additional 15 minutes, immediately followed by GC-MS analysis (see below). Results were unchanged when longer equilibration or exposure times were used.

The second method, dynamic headspace trapping (Raguso & Pellmyr, 1998), was used to quantify volatile compound emission rates during the first few hours after anthesis. Floral volatiles were concentrated within headspace bags (ca. 500 ml volume) and trapped on adsorbent cartridges using Supelco (Berwick, Pennsylvania, U.S.A.) personal air sampler vacuum pumps. Pasteur pipettes were packed with 100 mg of Super Q (80–100 mesh) adsorbent (Alltech Associates, Waukeegan, Illinois, U.S.A.) between plugs of quartz wool, and headspace air was pulled over the flowers and into the adsorbent trap at a flow rate of ca. 250 ml/min. Fragrance was collected for 6–8 hr. after anthesis (dusk in all taxa) in a protected area outside the greenhouse. Additional scent collection was performed for *Oenothera acutissima* plants growing at the type locality (see Appendix 1). Trapped fragrance was eluted with 3 ml of high-purity hexane and stored at –20°C in Teflon-capped borosilicate glass vials. Before GC-MS analysis, we used a flow of gaseous N_2 to concentrate samples to 75 µl, then

added 5 µl of 0.03% toluene (16 ng) as an internal standard. One µl aliquots of each sample were injected into a Shimadzu GC-17A equipped with a Shimadzu QP5000 quadrupole electron impact MS (Shimadzu Scientific Instruments, Columbia, Maryland, U.S.A.) as a detector. All analyses were done using splitless injections on a polar GC column (diameter 0.25 mm, length 30 m, film thickness 0.25 µm (Econo Cap's carbowax coating, known as EC WAX); Alltech Associates), as described by Raguso et al. (2003a). SPME fibers were directly injected into the GC injection port at 240°C and were analyzed using the GC-MS parameters described above. Compounds were tentatively identified using computerized mass spectral libraries (Wiley Registry of Mass Spectral Data, National Institute of Standards and Technology, and Robert Adams' libraries (> 120,000 mass spectra)). Chiral GC was not available to us, so compounds with chiral carbons (e.g., α-pinene) were assumed to represent racemic mixes. Whenever possible, GC peak identities were verified using co-retention with known standards on both EC WAX and EC-5 GC columns. Peak areas were integrated using Shimadzu's Class-5000 software and were quantified by comparison with the internal standard. Total scent emission rates (per hour) were calculated as sums of all peak areas, converted to nanograms using the internal standard, and expressed per flower, and per gram fresh and dry floral mass.

COMPARISON OF SCENT COMPOSITION

We compared variation in fragrance profiles within and among taxa using the relative amounts of floral volatiles. For each individual, we calculated the proportion of total scent contributed by each compound and standardized these data to *z* scores, which have means of zero and variances of one. These values were used to compute a matrix of pairwise dissimilarity values between all individuals in the study (Euclidean distance). Pairwise dissimilarity values among conspecific (and co-occuring) individuals were compared to those between heterospecific (or disjunct) individuals using the Wilcoxon rank sum test (SPSS 11.5). This constituted a two-tailed test of the null hypothesis that mean ranks of pairwise dissimilarity values between taxa or populations did not differ from mean ranks of pairwise dissimilarity values within groups (see Levin et al., 2001, 2003b).

FACTOR ANALYSIS OF FLORAL PHENOTYPE

Ordination was used to determine whether combinations of floral attributes were characteristic for different section *Lavauxia* taxa. Floral morphological measurements including floral diameter, tube flare, floral depth, herkogamy, dry mass, and total number of scent compounds were normally distributed and were not transformed. Scent emission rates (ng scent per flower per hour) were calculated for the sums of all (1) nitrogenous, (2) ocimene-derived, and (3) linalool-derived compounds; these rates were then natural log-transformed ($y = \ln(x+1)$) for analysis. These data were combined for principal component analysis using the varimax rotation option (SPSS 11.5), in which factors with eigenvalues greater than unity were retained. Discriminant function analysis (SPSS 11.5) was then used to determine whether factor loadings could be used to correctly assign individuals to their source population or taxon.

POLLINATOR OBSERVATIONS FOR *OENOTHERA ACUTISSIMA*

Floral visitation to a natural population of *Oenothera acutissima* was observed at the type locality in Daggett Co., Utah, U.S.A. (Appendix 1). Several hundred plants were found in bloom along with flowering individuals of *Achillea* L., *Geum* L., *Iris* L., *Opuntia* Mill., and *Potentilla* L. species in a moist meadow at the margin of a *Pinus ponderosa* Douglas forest. Individual flowers were watched on the evenings of 11 June 2001 and 25–28 June 2003, for a total of 19 observer hours. Insect visitors were photographed and collected for identification when necessary. Voucher specimens remain in the possession of the lead author. To relate flower visitation to ambient conditions, light levels and temperatures were measured at the study site at one- to three-minute intervals. Light levels were measured in cd/m^2 with a highly sensitive silicon detector attached to a radiometer (International Light, IL1700). The time for sunset was obtained from the NASA database (http://aa.usno.navy.mil/).

Results

FLOWER SIZE AND COLOR

Flower size varied dramatically among taxa. The small, short-tubed flowers of *Oenothera flava* subsp. *flava* did not open fully in the greenhouse or the field. When buds were forced open on the evening of floral maturity (determined by color and firmness), the mean floral diameter was only 25 mm (Table 1). Flowers of *O. triloba* were twice this large on average, whereas flowers of *O. flava* subsp. *taraxacoides* and *O. acutissima* measured 60–85 mm in diameter. The nectar tubes (hypanthia) of all taxa are long compared with other North American flowers and vary markedly among taxa and populations (Fig. 1). Flowers of *O. flava* subsp. *taraxacoides* from Arizona were 1.1–1.7-

Table 1. Flower morphological measurements and nectar characteristics; plants from localities in Appendix 1 were grown under common greenhouse conditions. AZ, Arizona; NM, New Mexico; OA, *Oenothera acutissima*; OFF, *Oenothera flava* subsp. *flava*; OFT, *Oenothera flava* subsp. *taraxacoides*; OT, *Oenothera triloba*; v/v, volume per volume.

Measurement, mean ± SEM (range, N)	OA	OT	OFF	OFT	
				NM	AZ
Floral diameter (mm)	86.3 ± 1.1 (80–98, 21)	42.4 ± 0.9 (32–53, 28)	24.4 ± 0.8 (19–32, 19)	65.0 ± 2.2 (49–78, 14)	82.9 ± 2.2 (65–101, 18)
Corolla flare (mm)	7.7 ± 0.2 (6.5–9.0, 21)	4.2 ± 0.1 (3.0–5.5, 28)	2.8 ± 0.1 (1.5–3.5, 19)	7.3 ± 0.3 (6.0–8.0, 8)	7.4 ± 0.2 (6–8.5, 18)
Floral depth (mm)	96.7 ± 4.0 (75–135, 21)	82.3 ± 1.7 (66–97, 28)	57.8 ± 1.7 (43–71, 19)	166.1 ± 3.8 (150–186.5, 14)	177.5 ± 3.6 (155–203, 18)
Stamen length (mm)	128.1 ± 4.6 (100–171, 21)	97.9 ± 2.1 (80–116, 28)	66.7 ± 1.9 (51–87, 19)	190.4 ± 4.3 (170–216, 14)	201.8 ± 4.1 (176–238, 18)
Style length (mm)	141.5 ± 4.7 (113–184, 21)	98.4 ± 2.1 (82–116, 28)	63.7 ± 1.9 (51–72, 19)	196.6 ± 4.0 (177–227, 14)	212.3 ± 3.9 (184–247, 18)
Herkogamy (mm)	13.4 ± 0.7 (10–21, 21)	0.6 ± 0.3 (−2–4, 28)	−2.9 ± 0.7 (−9–3, 19)	6.2 ± 1.1 (0–13, 14)	10.5 ± 1.0 (0–16, 18)
Nectar standing crop (μL)	9.7 ± 1.3 (3.5–15, 8)	11.5 ± 2.0 (4.3–22, 10)	2.3 ± 0.6 (0.8–5.0, 11)	11.2 ± 1.3 (6.2–15.9, 6)	40.9 ± 2.9 (26–56, 9)
Nectar concentration (% v/v)	25.4 ± 0.8 (19–30, 13)	27.4 ± 0.8 (22–31, 10)	25.9 ± 0.9 (23.5–32, 8)	26.6 ± 0.7 (23–30, 12)	25.2 ± 0.9 (16.5–31.5, 17)
Fresh mass (g)	1.26 ± 0.02 (1.21–1.29, 3)	0.32 ± 0.02 (0.19–0.44, 14)	0.17 ± 0.01 (0.14–0.25, 12)	1.55 ± 0.07 (1.22–1.81, 8)	1.92 ± 0.07 (1.63–2.38, 9)
Dry mass (g)	0.14 ± 0.01 (0.12–0.16, 3)	0.04 ± 0.003 (0.02–0.06, 14)	0.02 ± 0.001 (0.02–0.03, 12)	0.18 ± 0.006 (0.16–0.21, 8)	0.23 ± 0.01 (0.16–0.35, 9)

fold larger than *O. flava* subsp. *taraxacoides* from New Mexico in corolla diameter, nectar tube length, and anther-stigma separation. Stigmas of *O. acutissima* flowers were exserted the greatest mean distance beyond the anthers (positive herkogamy: 13.4 mm), whereas flowers of *O. flava* subsp. *flava* were consistently negatively herkogamous, with the open stigma lobes positioned 3 mm below the dehiscing anthers, on average. Flowers of *O. triloba* varied continuously from negative to positive herkogamy (Table 1).

All taxa produced flowers that were yellow to the human eye. Flowers of *Oenothera triloba* appeared to be the least saturated yellow (Fig. 1) but were not less reflective than other taxa in yellow wavelengths (Fig. 2). The inner petal surfaces of *O. acutissima* flowers were uniformly reflective above 500 nm but absorbed light of shorter wavelengths, including ultraviolet (UV) (Fig. 2). In all other taxa, the proximal portion of each petal (i.e., the central region of the corolla) absorbed UV, whereas the distal petal regions reflected strongly from 350 to 380 nm, creating a pattern of UV contrast at the flower's center (Fig. 2). Dissection of buds revealed this pattern to be present at least 24 hr. before anthesis (data not shown).

NECTAR VOLUME AND CONCENTRATION

Nectar volumes per flower varied considerably between taxa and populations, with means ranging from 2.3 μl in the cleistogamous *Oenothera flava* subsp. *flava* to more than 40 μl in *O. flava* subsp. *taraxacoides* from Arizona (Table 1). Surprisingly, the flowers of *O. triloba* produced nectar volumes comparable to those of the much larger flowers of *O. acutissima* and *O. flava* subsp. *taraxacoides* from New Mexico. On the other hand, flowers of *O. flava* subsp. *taraxacoides* from Arizona produced at least four-fold more nectar than those of any other taxon studied (Table 1), despite being grown on the same greenhouse bench. Nectar sugar concentrations and ranges were nearly identical (25%–27.5% volume per volume dissolved sucrose equivalents) among all taxa, including *O. flava* subsp. *flava*.

FLORAL SCENT EMISSION RATES AND CHEMICAL COMPOSITION

Odor emission rates varied dramatically, with large-flowered taxa producing up to 20-fold more scent per flower than small-flowered taxa (Fig. 3). Flowers of *Oenothera flava* subsp. *flava* and *O. triloba* had the lowest emission rates and were only half as strongly scented as large-flowered taxa even when standard-

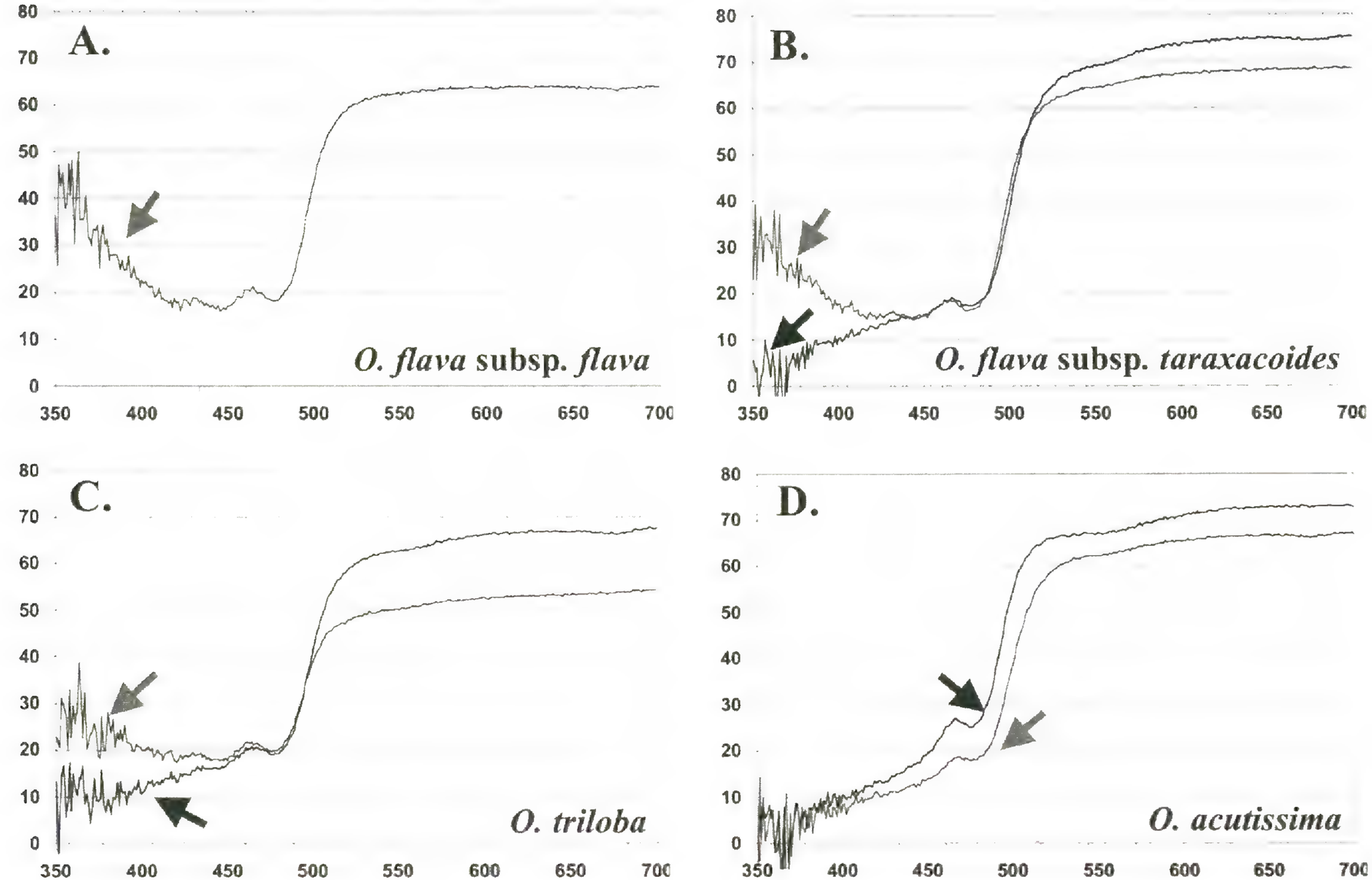

Figure 2. Spectral reflectance curves for upper (adaxial) petal surfaces, from 350–700 nm in wavelength. The y-axis represents percent reflectance, compared to white standard. In each panel, the curve marked by the grey arrow shows reflectance over a 1 cm diameter (0.78 cm^2 area) disc within the proximal (basal) region of the corolla lobe, and the curve marked by the black arrow shows reflectance over a similar area within the distal region of the petal. Petals of *Oenothera flava* subsp. *flava* (panel A) were too small to dissect in this way. *Oenothera flava* subsp. *taraxacoides*, *O. triloba*, and *O. acutissima* are shown in panels B, C, and D, respectively.

ized for fresh and dry floral mass. Although flowers of *O. flava* subsp. *taraxacoides* from Arizona were 20% larger than those from New Mexico, the latter were twice as strongly scented as the former, both per flower and per unit floral mass (Fig. 3). Emission rates of greenhouse-grown *O. acutissima* plants were comparable to those of *O. flava* subsp. *taraxacoides* from New Mexico but were less than half as strong as those measured from *O. acutissima* in the field (Fig. 3).

Fifty-four volatile organic compounds were detected in floral headspace, of which 32 were identified using known standard compounds or commercially available essential oil blends. Scent compounds represented several biosynthetic categories, including mono- and sesquiterpenoids, benzenoid (aromatic) compounds, fatty acid derivatives and, especially, nitrogenous compounds derived from amino acids (Appendix 2). The nitrogenous aldoximes, nitro- and nitrile compounds derived from valine, leucine, isoleucine, and phenylalanine (Fig. 4), were present in all taxa, whereas sesquiterpenoids were limited to *Oenothera acutissima*. As a class, the nitrogenous compounds accounted for 67%–96% of total scent emissions in all *O. flava* populations, but only 9.3% and 37% of emissions from *O. triloba* and *O. acutissima*, respectively. The scents of *O. triloba* and *O. acutissima* were dominated (62%–72% of emissions) by monoterpenoids (Fig. 5). Two additional nitrogenous compounds, nicotinic acid methyl ester and 1-pyrroline (Fig. 4), were present in all taxa surveyed. The monoterpene trans-β-ocimene constituted 30%–44% of total emissions in most taxa but was a minor scent component (< 4%) in *O. flava* subsp. *flava* and *O. flava* subsp. *taraxacoides* from Arizona (Fig. 5). SPME analysis revealed that methyl benzoate and 1-pyrroline were present exclusively in the floral nectar of all taxa, whereas most other compounds were emitted by petals and other flower parts (data not shown).

Taxa with large, putatively outcrossing flowers produced the most complex fragrances, with 24–27 compounds in *Oenothera flava* subsp. *taraxacoides* and 34–37 compounds in *O. acutissima*, including the fruity-scented isoamyl alcohol and three of its esters (Appendix 2, Fig. 6). In contrast, the small, putatively autogamous flowers of *O. flava* subsp. *flava* and *O. triloba* produced the least chemically complex odors (13–21 compounds). This result is not an artifact of too little floral tissue being used for odor analysis, as some samples included up to five flowers. When the number of scent compounds of *O. triloba* was regressed against total floral mass per sample, odor complexity was not significantly correlated with sample floral mass (linear regression, $R^2 = 0.001$, $P = 0.94$).

Floral scent blends generally were taxon-specific (Table 2). Fragrance composition between isolated populations of *Oenothera flava* subsp. *taraxacoides* was similar, except for the presence of trace amounts (< 1% of total emissions) of linalool and its furanoid oxides in plants from Arizona and substantial amounts (nearly 30%) of trans-β-ocimene in those from New Mexico. The exception to the pattern of taxon-specific odors was *O. acutissima*, in which the presence of two distinct scent phenotypes or "chemotypes" resulted in sufficient within-taxon variation such that the floral fragrance produced by conspecific individuals was not significantly more similar than that produced by flowers of different species (Table 2). The floral scent of individual plants was dominated either by linalool or by trans-β-ocimene, to the near exclusion of the other compound. Three of eight field-collected fragrance samples were linalool-dominated (mean ± SEM = 40.7 ± 4.2% of total emissions) with little trans-β-ocimene (6.0 ± 3.0%), whereas trans-β-ocimene was the dominant component of the remaining five plants (62.4 ± 2.6%), in which linalool was nearly absent (0.2 ± 0.06%). Similar patterns were observed in greenhouse-grown plants, none of which emitted large amounts of both compounds. Compositional differences between greenhouse- and field-grown *O. acutissima* were restricted to minor, derivative components. Field-grown plants produced unique linalool-derived compounds, whereas greenhouse-grown plants produced putative ocimene-derivatives, increased amounts of β-caryophyllene, and related sesquiterpenes not detected in the fragrance of field-grown plants.

FACTOR ANALYSIS OF FLORAL ADVERTISEMENTS AND REWARDS

Principal component analysis identified two factors with eigenvalues greater than unity, accounting for 85.8% and 12.5% of total sample variance, respectively. Floral dimensions above the nectar tube (floral diameter, corolla flare, and herkogamy) were highly correlated and loaded positively on factor 1, along with the total number of scent compounds and emission rates of linalool- and ocimene-derived scent compounds (Table 3, Fig. 7). This dimension clearly separated *Oenothera acutissima* from *O. flava* subsp. *flava* and *O. triloba* in floral phenotype space, and separated most individuals of *O. flava* subsp. *taraxacoides* from Arizona and New Mexico into their respective populations (Fig. 7). Floral depth, dry floral mass, and the emission of nitrogenous scent compounds had the strongest loading scores on factor 2, for which the only character with negative loadings was the emission of linalool and related scent compounds (Table 3). Floral diameter, herkogamy,

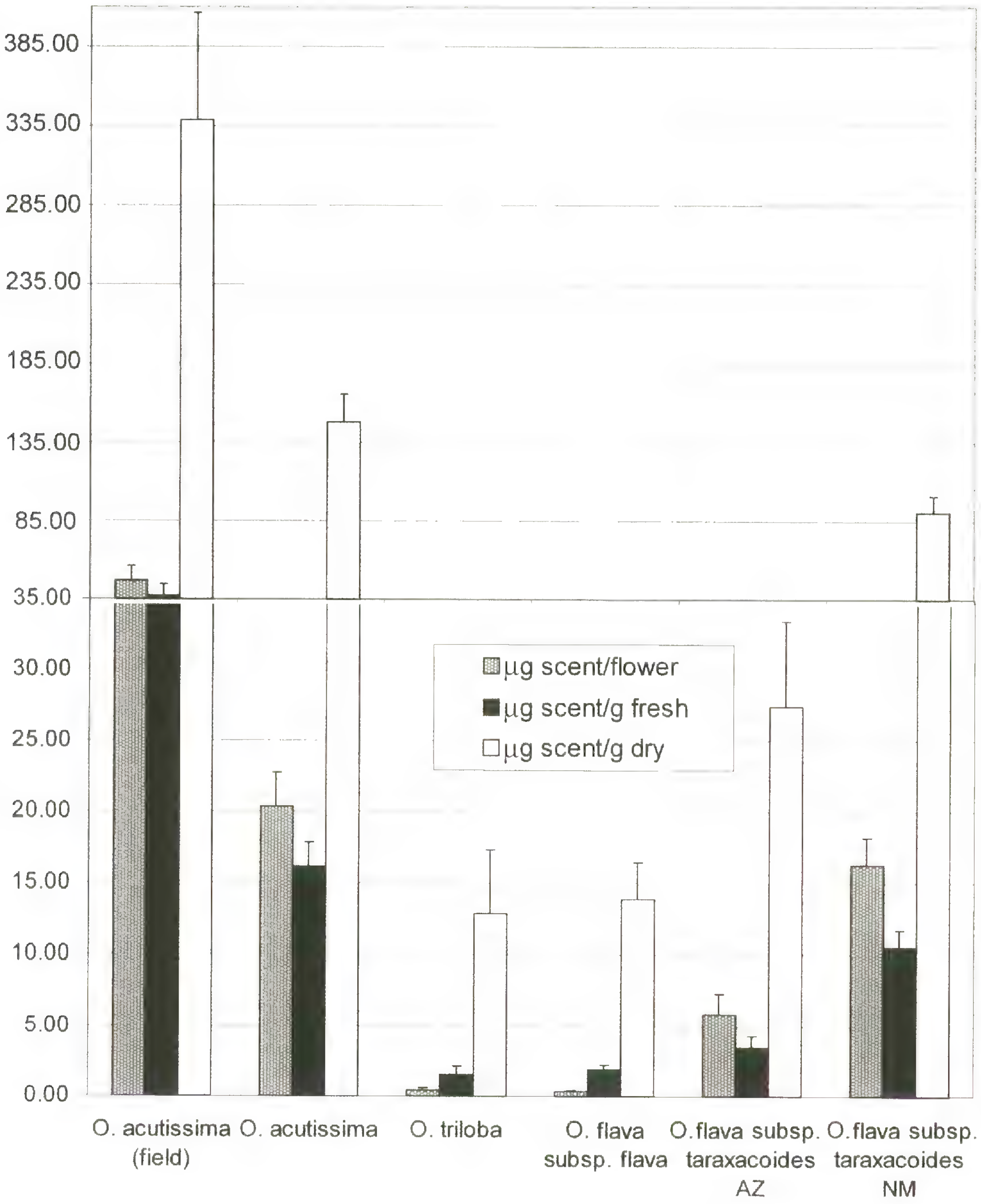

Figure 3. Hourly emission rates of floral scent, per flower (stippled bars), per gram fresh floral mass (dark), and per gram dry floral mass (light). In order to accommodate the substantial range of variation, the y-axis is discontinuous. Note the similarity in emission rates between the two putatively autogamous taxa (*Oenothera triloba* and *O. flava* subsp. *flava*) and between the outcrossing taxa (*O. acutissima* and *O. flava* subsp. *taraxacoides*). Also note the two-fold increase in emissions from *O. acutissima* collected in its native habitat. All other collections were made from plants grown in a common greenhouse setting.

and corolla flare loaded positively on both factors, with larger loadings on factor 1. Factor 2 clearly separated *O. flava* subsp. *taraxacoides* from all other taxa, and the combined factors separated the small-flowered taxa, *O. triloba* and *O. flava* subsp. *flava*, from each other (Fig. 7). Discriminant function analysis correctly assigned 95% of the 44 individuals sampled to the correct taxon and population except for

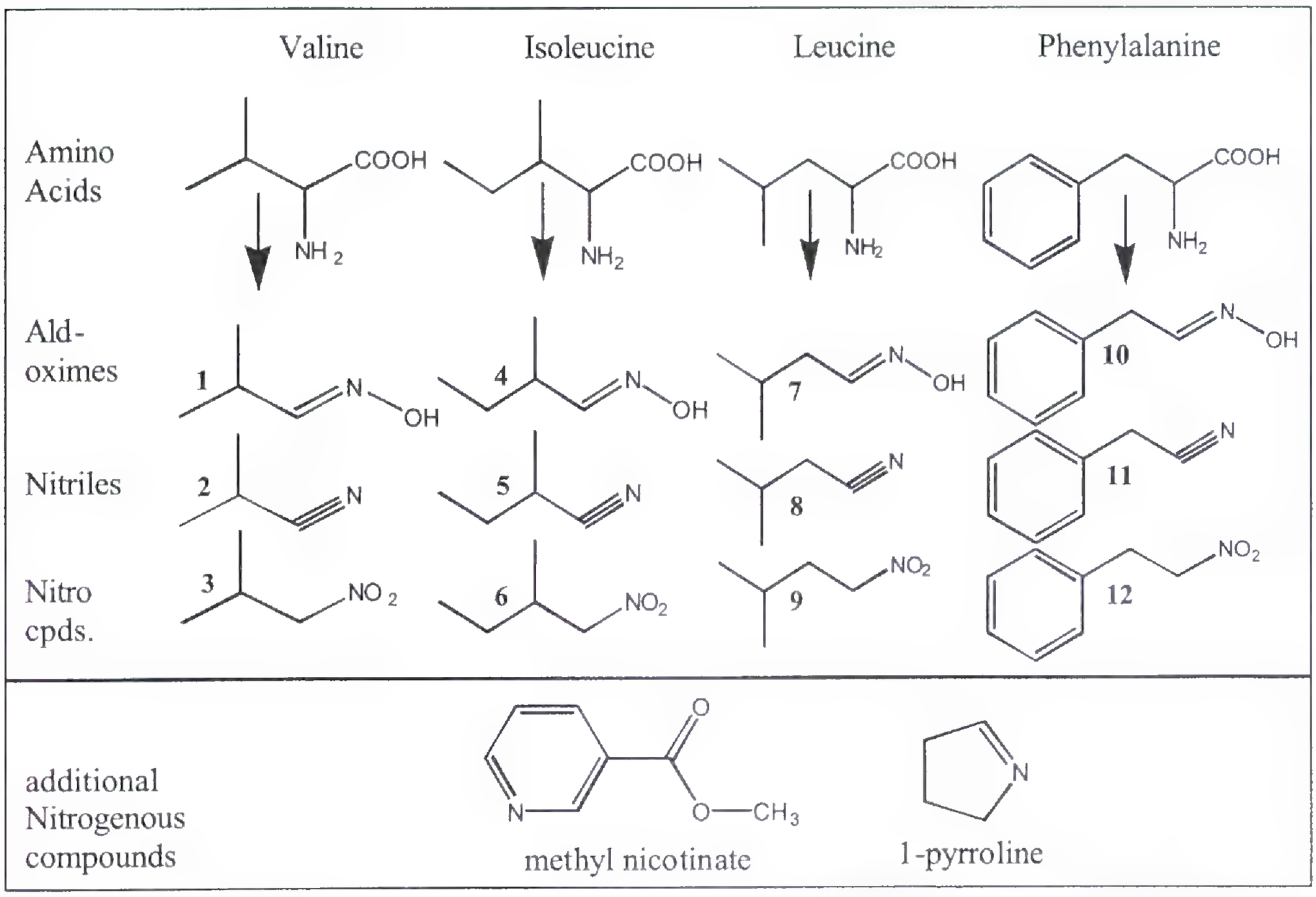

Figure 4. Nitrogenous volatile compounds prominent in the floral scent of *Lavauxia* species. The vertical series in the upper panel shows the derivation of aldoximes, nitriles, and nitro-compounds (in descending order) from their putative amino acid precursors, after Kaiser (1993). Numbered compounds are (1) 2-methylpropanaldoxime, (2) 2-methylpropylnitrile, (3) nitro-2-methylpropane, (4) 2-methylbutyraldoxime, (5) 2-methylbutyronitrile, (6) nitro-2-methyl butyrate, (7) 3-methylbutyraldoxime, (8) 3-methylbutyronitrile, (9) nitro-3-methyl butyrate, (10) phenylacetaldoxime, (11) phenylacetonitrile, and (12) nitro-2-phenylethane. Note that each aldoxime skeleton occurs in syn- and anti- isomers (not shown), and that compounds 2 and 12 were not detected in this study. Lower panel shows structures of methyl nicotinate, present in all *Oenothera* sect. *Lavauxia* but in few other *Oenothera* species, and 1-pyrroline, present in the nectar of all *Oenothera* studied to date.

O. flava subsp. *taraxacoides*, for which one plant in each population was incorrectly assigned to New Mexico or Arizona populations.

FLORAL BIOLOGY OF *OENOTHERA ACUTISSIMA*

Flowers of *Oenothera acutissima* opened in the evening, from 30 minutes before to 15 minutes after sunset. In 2001 and 2003, flowers were visited frequently at dusk by crepuscular hawkmoths, including *Hyles lineata* Fab., *Sphinx chersis* Hubner, *Sphinx vashti* Strecker, and *Manduca quinquemaculata* Haworth (Table 4, Fig. 8). On 25 June 2001, a mean of 0.9 visits per flower (N = 13 watched flowers) was observed during a 30-minute period, whereas on 27 June 2001, a mean of 1.3 visits per flower (N = 14 flowers) occurred during a 20-minute period (Table 4). *Hyles lineata* and the *Sphinx* species first arrived at flowers of *O. acutissima* 30 minutes after sunset (20:45 MST) and continued to forage sporadically until 22:00 hr., when observations ended due to darkness. *Manduca quinquemaculata* individuals were not seen until 40 minutes after sunset and continued visiting flowers until observations ceased. During hawkmoth visitation, ambient temperatures dropped from 15°C to 10°C, and light intensity decreased from 120 cd/m^2 at sunset to 1.25 cd/m^2 at first *H. lineata* visit, and 0.06 cd/m^2 at first *M. quinquemaculata* visit. *Hyles lineata* and *Sphinx* moths visibly removed large amounts of pollen on their head and legs (Fig. 8D), whereas *M. quinquemaculata* carried pollen on the legs and the extended proboscis (Fig. 8F). Female *H. lineata* moths oviposited on leaves of *O. acutissima* between flower visits, and several larvae of different developmental stages were observed eating flower buds in this population. Flowers remained open and bright yellow until 10:00 hr. on the following day before losing turgor and turning deep brick red. Small halictid bees, *Evyleus (Lasioglossum) aberrans* Crawford, collected pollen from individual anthers within an hour after sunrise but did not frequently touch the extended stigmas (Fig. 8B). Mule deer (*Odocoileus hemionus* Rafinesque) were abundant in 2003 and browsed

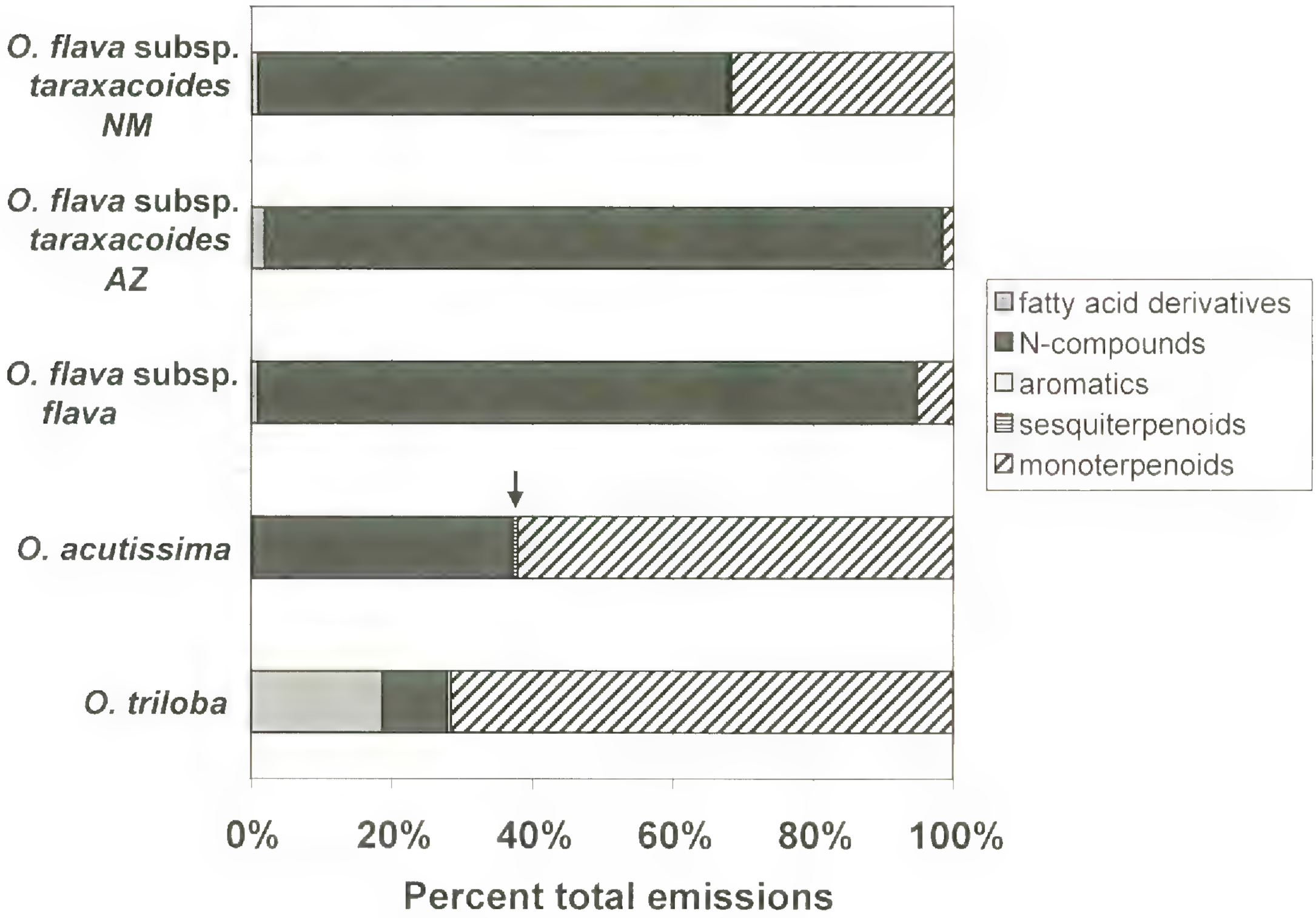

Figure 5. Contributions (relative %) of different compound classes to floral scent emissions in *Oenothera* sect. *Lavauxia* taxa. Nitrogenous compounds (dark solid bars) are dominant fragrance components in all *O. flava* populations, whereas monoterpenoids (striped bars), specifically trans-β-ocimene, dominate the fragrances of *O. acutissima* and *O. triloba*, and aromatic (white) and sesquiterpenoid (stippled, see arrow) compounds are present in trivial amounts (barely visible between nitrogenous and monoterpenoid bars). The seemingly large (ca. 20%) contribution of fatty acid derivatives (cis-3-hexenol-related compounds) to *O. triloba* emissions reflects this taxon's relatively weak floral scent.

heavily upon buds and newly opening flowers in the evening and upon flowers that remained open in early morning.

Discussion

Floral Advertisements: Visual and Olfactory Display

Floral visual cues and scent chemistry are relatively conserved among North American species of *Oenothera* section *Lavauxia*. All taxa studied have yellow flowers that, with the exception of *O. acutissima*, have contrasting patterns of distal UV-reflectance and proximal UV-absorbance on the upper petal surfaces (Fig. 2). This pattern creates a concentric "target" of UV contrast previously documented for other yellow-flowered *Oenothera* species (Dement & Raven, 1974). It is unclear whether this target is a phylogenetically conserved trait in the lineage that includes section *Lavauxia* or an adaptation for proboscis placement by hawkmoths, as suggested by Kawano et al. (1995) for *O. biennis* L. and *O. glazioviana* Micheli naturalized to Japan. Visual contrast (including UV wavelengths) is an important orientation or landing cue for several bee species (Jones & Buchmann, 1974; Chittka et al., 1994) and may indicate historical and/or present pollination by crepuscular or matinal bees, as documented for other yellow-UV–colored *Oenothera* species (Towner, 1977; Barthell & Knops, 1997). On the other hand, the diurnal hawkmoth *Macroglossum stellatarum* L. uses visual contrast to position itself before a flower and prefers flowers with centered marks of contrast over those with off-center or no marks (Kelber, 1997). If such preferences are shared by crepuscular and nocturnal hawkmoths, the UV patterns in section *Lavauxia* species would provide appropriate visual contrast.

Floral scent chemistry also is relatively constrained among North American *Oenothera* sect. *Lavauxia*, with most taxa producing nested subsets of the odor blend emitted by *O. acutissima* (Fig. 6). Four intriguing patterns emerge from our chemical data set. The first is the dominance of amino acid–derived nitrogenous odors, particularly the four aldoxime isomers derived from leucine and isoleucine (Figs. 4, 5). These compounds are produced by a number of

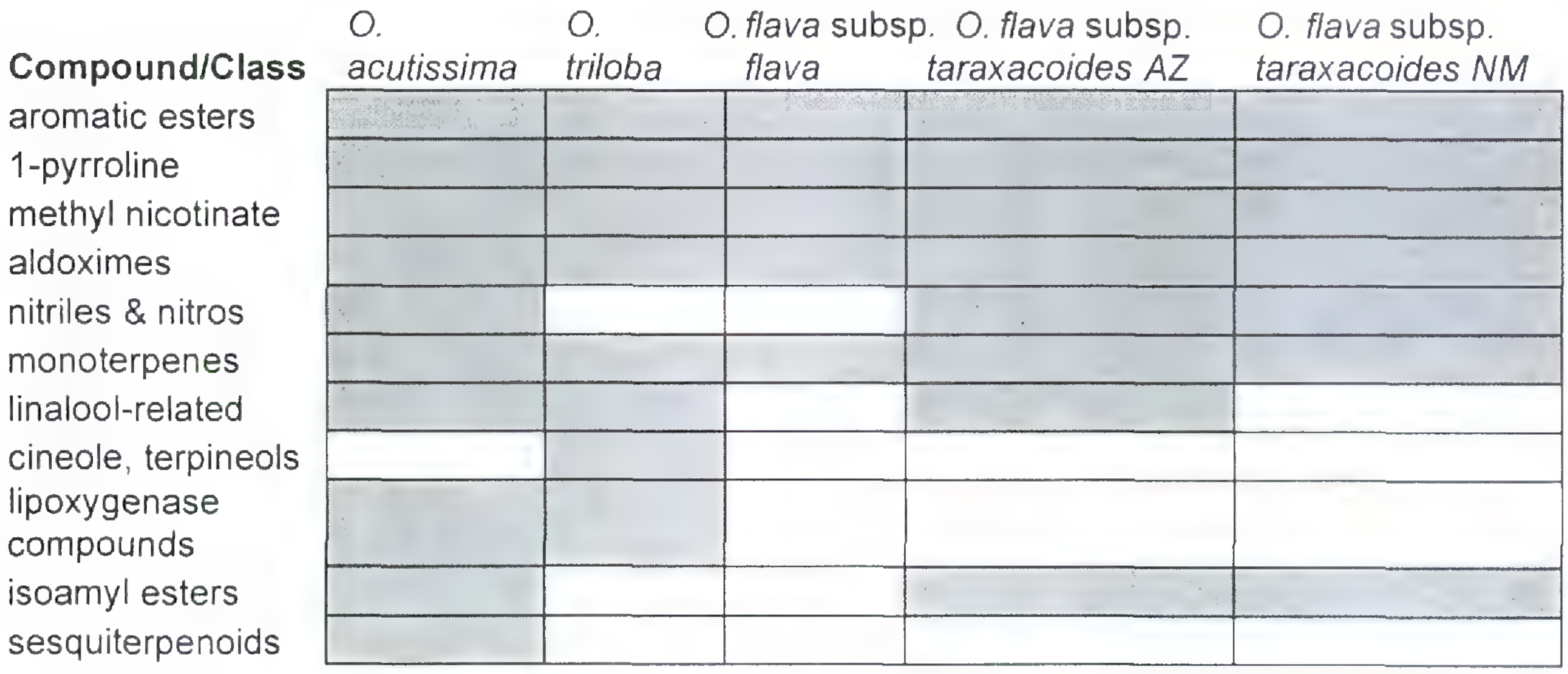

Figure 6. Nested representation of the presence/absence of specific compound classes in the floral scent of *Oenothera* sect. *Lavauxia* species. Sesquiterpenoids and oxygenated cyclohexane monoterpenoids (1,8-cineole, α-terpineol) are autapomorphic in *O. acutissima* and *O. triloba*, respectively.

night-blooming, obligately outcrossing species of *Oenothera*, including *O. primiveris* A. Gray (Raguso, 2004), *O. xylocarpa* Coville, *O. howardii* (A. Nelson) W. L. Wagner, and most species in section *Pachylophus* (R. A. Raguso, unpublished data). Aldoximes also are frequently produced by night-blooming, hawkmoth-pollinated flowers of diverse plant families, such as *Datura* L. and *Nicotiana* L. (Solanaceae), *Angraecum* Bory (Orchidaceae), and *Hedychium* J. Koenig (Zingiberaceae) species (Kaiser, 1993; Knudsen & Tollsten, 1993; Raguso et al., 2003a, b; Raguso, 2004). However, aldoximes are absent from two lineages of hawkmoth-pollinated Nyctaginaceae (*Acleisanthes* A. Gray and *Mirabilis* L.), in which the only nitrogenous floral volatiles are the tryptophan-derived indole and methyl anthranilate (Levin et al., 2001, 2003b). Behavioral experiments are needed to determine the function (if any) of aldoximes and their metabolites in the scents of night-blooming flowers, and whether other nitrogenous volatiles (e.g., indole) play similar roles in hawkmoth attraction to flowers.

The second notable result is the presence of methyl benzoate and 1-pyrroline in floral nectar throughout section *Lavauxia* and other sections of *Oenothera* (Raguso, 2004). Scented nectar occurs among disparate angiosperm families, but its role in plant reproductive ecology is poorly understood (Raguso, 2004). Methyl benzoate is widely distributed among floral scents (Knudsen et al., 1993) and elicits strong antennal responses from *Manduca sexta* L. and other

Table 2. Variation in scent profiles based on dissimilarity between pairs of individuals. Mean, minimum and maximum percent dissimilarity (Euclidean distances), and number of pairwise comparisons (N) within and between taxa or populations are shown. Mean ranks were compared using the Wilcoxon rank sum test for independent samples. Besides field data for *Oenothera acutissima*, all data were collected from a common greenhouse study. Note that between-group dissimilarity measures are significantly greater than within-group values overall, and for all taxa studied except *O. acutissima*. AZ, Arizona populations; NM, New Mexico populations.

	Raw mean % dissimilarity (range) [N]		Mean rank			
	Within-group	Between-group	Within-group	Between-group	*z* Score	*P*
All species	3.54 (0.03–9.83) [167]	7.35 (1.10–10.71) [653]	25.4	88.5	−10.7	<0.001
O. acutissima (field)	5.33 (0.63–9.78) [28]	6.68 (0.39–10.40) [328]	8.0	22.0	−1.9	0.059
O. acutissima	5.14 (0.57–9.83) [10]	6.34 (1.10–10.63) [180]	2.7	6.7	−2.0	0.047
O. triloba	3.92 (0.03–7.55) [55]	7.49 (1.10–10.71) [400]	21.2	29.5	−4.7	<0.001
O. flava subsp. *flava*	1.68 (0.63–2.79) [21]	7.46 (1.14–10.64) [294]	0.0	11.0	−4.0	<0.001
O. flava subsp. *taraxacoides* (NM)	3.48 (0.78–9.03) [36]	6.03 (1.14–10.61) [330]	9.9	21.0	−4.0	<0.001
O. flava subsp. *taraxacoides* (AZ)	3.65 (0.77–7.05) [45]	8.23 (2.45–10.71) [390]	0.0	23.0	−5.8	<0.001

hawkmoths (Raguso et al., 1996; Fraser et al., 2003). Its potential as a gustatory stimulant or, possibly, an anti-microbial nectar component deserves further study. Wagner (1981: 155) described the fragrance of *O. acutissima* flowers as a "light, semen-like odor." This is undoubtedly due to 1-pyrroline, which bears a sharp, semen-like odor (Amoore et al., 1975), is a known derivative of the amino acid proline, and is produced by rearrangement of the polyamine putrescine (Robacker, 2001). The narrowness and extreme depth of the hypanthium in most *Lavauxia* taxa, combined with the mode of pollen dehiscence in viscin threads, argues against proline contamination (via pollen) as a source of this nectar component. The biological function of 1-pyrroline in *Oenothera* nectar is unknown, but it is present in the nectars of other long-tubed night-blooming flowers (e.g., *Datura wrightii* Regel, *Ipomoea alba* L.; R. A. Raguso, unpublished data) and is emitted by the inflorescences of many plant species that produce numerous,

Table 3. Principal component analysis of floral metrics and scent emissions. Loadings are given for two factors accounting for 98.3% of total sample variance. Loadings greater than ± 0.5 are shown in boldface. Values for scent were natural log (ln) transformed for the analysis.

	Loadings	
Floral Character	Factor 1 (85.8%)	Factor 2 (12.5%)
Floral depth	0.164	**0.987**
Floral diameter	**0.881**	0.468
Corolla flare	**0.731**	**0.598**
Herkogamy	**0.788**	0.454
Dry floral mass	0.421	**0.836**
Number of scent compounds	**0.709**	0.376
ln [Nitrogenous scent]	0.059	**0.661**
ln [Ocimene-derived scent]	**0.521**	0.122
ln [Linalool-derived scent]	**0.756**	−0.229

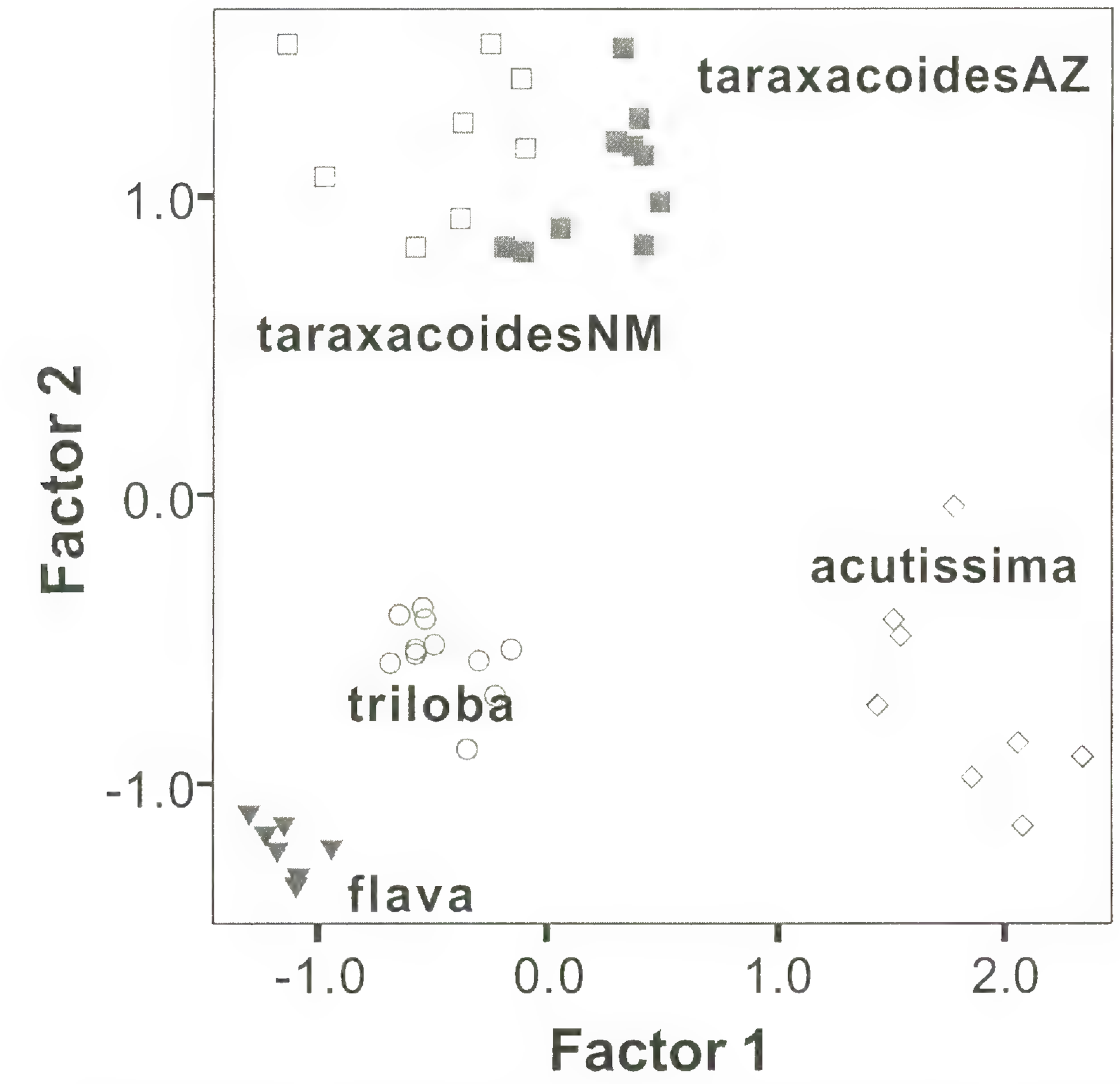

Figure 7. Scatterplot of loadings on factors 1 and 2 from different *Oenothera* sect. *Lavauxia* taxa. These variables clearly separate all taxa, including the two populations of *O. flava* subsp. *taraxacoides*, as 98% of individuals are correctly assigned to their population of origin using discriminant function analysis (see text).

Table 4. Number of observed visits to flowers of *Oenothera acutissima* at type locality, Uinta Mountains, Utah. Total flowers were not counted on days indicated by*.

Insect	11 June 2001	25 June 2003	27 June 2003	28 June 2003	Totals
Lepidoptera, Sphingidae					
Hyles lineata	4	8	1	0	13
Manduca quinquemaculata	0	3	3	2	8
Sphinx vashti and *S. chersis*	15	2	11	4	32
Hymenoptera, Halictidae					
Evyleus aberrans	5	0	0	0	5
Totals	24	13	15	6	58
Mean visits per flower	*	0.9	1.3	*	
Cumulative observer hours	3	10	5	1	19

small, white flowers in spikes or umbels (Raguso, 2006).

The third notable finding was the presence of the nitrogenous ester, methyl nicotinate, in all taxa studied, regardless of flower size or pollination system. We have identified this compound in other night-blooming plants (e.g., *Selinocarpus chenopodioides* A. Gray (Nyctaginaceae), Levin et al., 2001; *Nicotiana suaveolens* Lehm. (Solanaceae), Raguso et al., 2003a), but its biological function, if any, has not been examined in the context of pollination. Methyl nicotinate may represent a biosynthetic artifact if nicotinic acid is unusually common in the petals of *Lavauxia* flowers and is converted to a volatile ester by a biosynthetic enzyme that normally uses another compound as a substrate (see D'Auria et al., 2002). Given the absence of methyl nicotinate from most other hawkmoth-pollinated *Oenothera* studied to date (R. A. Raguso, unpublished data) and its presence in all North American *Lavauxia*, we consider this compound to be a phylogenetically informative character.

The fourth noteworthy result was the identification of two "chemotypes" dominated by either trans-β-ocimene or linalool among individuals of *Oenothera acutissima*. Clearly delineated linalool- and ocimene-dominated "chemotypes" were not observed in other taxa. No *O. acutissima* plants of intermediate phenotype were found, but field collections may have been too few to detect such phenotypes if they are rare. Both scent morphs have compounds that are associated with sphingophily in other plant groups (Kaiser, 1993; Knudsen & Tollsten, 1993). Similarly distinct chemotypes have been observed for hawkmoth-pollinated *Platanthera* Rich. orchids in Scandinavia, in this case dominated by trans-β-ocimene, linalool, or geraniol (Tollsten & Bergström, 1993). Distinct chemotypes likewise are present in *O. caespitosa* Nutt. in the Great Basin Desert of North America (R. A. Raguso, unpublished data). The adaptive importance of intraspecific variation in floral scent is poorly understood in general (Azuma et al., 2001; Knudsen, 2002; Dötterl et al., 2005), and determination of the biological significance, if any, of discrete scent chemotypes in *Oenothera* will require manipulative behavioral and ecological experiments.

COVARIATION BETWEEN FLORAL PHENOTYPE AND PUTATIVE MATING SYSTEM

North American members of *Oenothera* section *Lavauxia* exhibit remarkable variation in quantitative aspects of floral phenotype: flower size, hypanthium length, nectar volume, and fragrance intensity. These traits varied consistently in plants grown under common garden/greenhouse conditions, along a continuum from small, weakly scented, putatively autogamous flowers (*O. flava* subsp. *flava*, *O. triloba*) to large, strongly scented, putatively outcrossing flowers (*O. acutissima*, *O. flava* subsp. *taraxacoides*). Specific combinations of floral characters clearly distinguish most of these entities from each other using ordination techniques (Fig. 7). The least clearly separated populations (*O. flava* subsp. *taraxacoides* from Otero Co., New Mexico, and Apache Co., Arizona) differ measurably in additional floral characters (Table 2, Fig. 6).

Olfactory and visual floral displays are dramatically reduced in taxa for which pollinator attraction is unnecessary. Floral scent in putatively autogamous taxa is two orders of magnitude less intense than in outcrossing taxa, even when floral size differences are standardized (Fig. 3). Thus, reduction of odor in *Oenothera flava* subsp. *flava* and *O. triloba* is not merely a consequence of scaling differences in flower size and probably involves regulatory elements of fragrance biosynthetic pathways (e.g., Raguso & Pichersky, 1999). However, chemical composition remains relatively similar among taxa (Figs. 5, 6). The absence of minor scent compounds (nitriles, nitro-compounds, and isoamyl esters) in small-flowered species is correlated with greatly reduced absolute

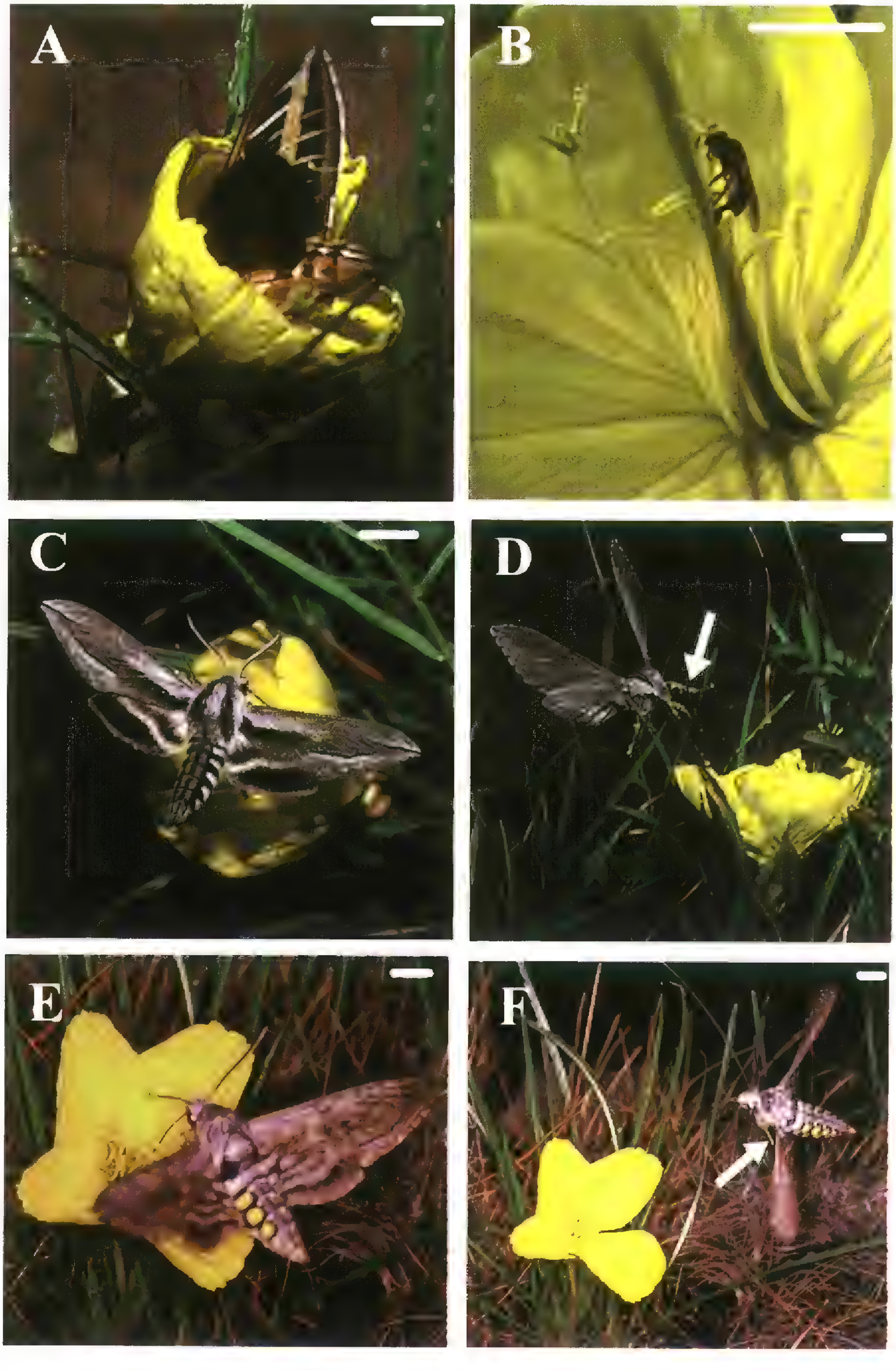

Figure 8. Floral visitors to *Oenothera acutissima* at its type locality in the Uinta Mountains, Daggett Co., Utah. Hawkmoths, including *Hyles lineata* (A), *Sphinx vashti* (C, D), and *Manduca quinquemaculata* (E, F), visit newly opened flowers at dusk. Note the yellow pollen adhering to the moth's legs in D and F (arrows). In panel B, a small *Evyleus* (*Lasioglossum*) *aberrans* bee strips pollen from an anther. Photos in A–D by R. A. Raguso, photos in E, F by M. Pfaff. All scale bars = 1 cm.

amounts of structurally similar aldoximes (Fig. 6), from which they may be derived. Thus, downregulation of biosynthetic enzymes and/or substrate flux (e.g., amino acids), rather than null mutations in specific pathway steps, are the most likely proximate mechanisms for scent reduction in small-flowered section *Larauxia* taxa (see Dudareva et al., 2000). Further physiological and ecological experiments will

be required to determine whether resource allocation costs or herbivore-mediated selection (e.g., Mothershead & Marquis, 2000) are the ultimate selective forces driving reduced floral display in *O. flava* subsp. *flava* and *O. triloba*.

In contrast, the qualitative aspects of floral phenotype—color, nectar, and overall scent chemistry—were quite similar among taxa, regardless of flower size or mating system. All taxa produced floral nectar with mean sugar concentrations within the range of diverse hawkmoth-pollinated flowers (Cruden et al., 1983; Haber & Frankie, 1989; Hodges, 1995; Raguso et al., 2003b); only the cleistogamous flowers of *Oenothera flava* subsp. *flava* produced nectar volumes that were likely too small to be rewarding (Table 2). Indeed, the relatively high nectar volumes in small-flowered *O. triloba* suggest the potential for a mixed mating system, rather than strict autogamy, and indicate the need for detailed field studies of this species across its range. These patterns suggest that evolutionary reversals from small autogamous flowers to large, outcrossing flowers may be easily accomplished in section *Lavauxia*, because the fundamental components of floral advertisement and reward remain intact in small-flowered taxa (see Armbruster, 1993).

EXTREME FLORAL VARIATION WITHIN *OENOTHERA FLAVA*

Our study populations of *Oenothera flava* represent the extreme large and small floral phenotypes known for that species. However, Wagner (1986) described continuous floral variation among populations of *O. flava* subsp. *flava* and *O. flava* subsp. *taraxacoides* across western North America, encompassing a range of variation similar to that presented here for the entire section *Lavauxia*. Flowers with intermediate morphology might represent mixed mating systems in which both self-pollination and hawkmoth-mediated outcrossing occur. Ashman and Schoen (1994) found that *O. flava* with intermediate-sized flowers in western Colorado accrued 82% and 97% of male and female function, respectively, within hours of anthesis through hawkmoth visitation and, presumably, self-pollination. Variation in the degree of self-pollination must be quite high in *O. flava*, as has been documented for a related species with a similar degree of variation in floral characters, *Calylophus serrulata* (Nutt.) P. H. Raven (Cruden & Lyon, 1989). Given the broad geographic range of *O. flava* and the distances between isolated, mountaintop populations of *O. flava* subsp. *taraxacoides* in Arizona and New Mexico, U.S.A., and Chihuahua, Mexico, it is unclear whether populations of *O. flava* subsp. *taraxacoides* have evolved independently as high-elevation, outcrossing ecotypes of *O. flava* subsp. *flava* or represent a distinct monophyletic entity that has dispersed among high-elevation habitats or has been isolated by changing climate (see Strand et al., 1996). The morphometric and chemical characters used in ordination provided only a modest degree of separation between individuals of *O. flava* subsp. *taraxacoides* from Arizona and New Mexico in phenotype space (Fig. 7). However, these populations also differed significantly in mean nectar volume ($t = 9.13$, $df = 11$, $P_{2\text{-tail}} < 0.001$) and total scent emission per dry mass ($t = 5.22$, $df = 11$, $P_{2\text{-tail}} < 0.001$, Fig. 3). High-elevation populations of *O. flava* subsp. *taraxacoides* clearly are differentiated to some degree, but it is unclear whether this reflects separate origins, genetic drift, or local pollinator adaptation (see Slentz et al., 1999; Boyd, 2002). Phylogeographic and population genetic analyses of these and other isolated populations will be required to better understand the systematic placement of *O. flava* subsp. *taraxacoides* with respect to other populations of *O. flava* and *O. acutissima*. However, flowers of *O. acutissima* were clearly differentiated from those of all taxa studied (Fig. 7) and produced the most complex odor blends (Appendix 2, Fig. 6). These differences in floral biology, combined with edaphic specialization, autapomorphic vegetative characters, and the poor viability (and sterility) of artificial hybrids between *O. acutissima* and *O. flava* (Wagner, 1981), cast doubt on Welsh's (1986) contention that *O. acutissima* is a form of *O. flava*.

HAWKMOTHS, DEEP FLOWERS, AND MONTANE HABITATS

We present the first pollinator observations for the rare, endemic species, *Oenothera acutissima*, taken at its type locality in the Uinta Mountains of Utah, U.S.A. The observed pattern of visitation by short- and long-tongued hawkmoths at dusk, followed by occasional visits of halictid bees the following morning, is consistent with observations of night-blooming *Oenothera* species in western North America over the past half century (Linsley et al., 1963a, 1963b, 1964; Gregory, 1964; Towner, 1977; Wagner et al., 1985). Although visitation rates generally were high (Table 4) and moths carried copious pollen on their appendages (Fig. 8), fruit set across the population was low. Capsules in section *Lavauxia* usually are indehiscent and accumulate among leaf scars from the previous year's growth (Wagner, 1986). However, in June 2001, Raguso found only three mature capsules on more than 100 plants. The densely crowded growth of *O. acutissima* in moist depressions, combined with low observed fruit set, suggests that plants may propagate clonally, or that capsules may abscise in this species. Further experiments are needed to determine the extent to which neighboring plants are

pollen compatible. Another possibility is that, despite pollinator abundance, the observed florivory by hawkmoth larvae and mule deer sharply reduced the reproductive success of *O. acutissima*. Plants were extremely cryptic when not in bloom, growing low among grasses, and flowers faded rapidly the next morning. Perhaps these aspects of plant form and flower duration have been shaped by selective pressure from herbivores, including browsing deer (see Herrera, 1993; Mothershead & Marquis, 2000).

Populations of *Oenothera flava* subsp. *taraxacoides* were collected from similarly mesic, montane habitats several hundred kilometers to the south. The nectar tubes of these plants, which sometimes exceed 20 cm in length, are among the deepest in the North American flora (Grant, 1983). A line of reasoning developed by Darwin (1862) and tested by Nilsson (1988) and Alexandersson and Johnson (2001) is that long nectar tubes or spurs result from extreme directional selection mediated by long-tongued hawkmoths. Gregory (1964) and Grant and Grant (1983a, b) predicted that a guild of widespread North American hawkmoths (*Agrius cingulata* Fab., *Manduca quinquemaculata*, *M. rustica* Fab., *M. sexta*) with proboscis lengths ranging from 8 to 12 cm are most likely to pollinate long-tubed, night-blooming flowers of the southwestern North American deserts, such as *Acleisanthes longiflora* A. Gray, *Mirabilis longiflora* L. (Nyctaginaceae), and *Datura wrightii* (Solanaceae) (Spellenberg & Delson, 1977; Raguso et al., 2003b). Unfortunately, few observations have been made on the floral visitors of *O. flava* subsp. *taraxacoides* at any one population. Gregory (1964) studied *O. flava* subsp. *taraxacoides* at its type locality in the Sacramento Mountains, New Mexico, and found that short-tongued (3–4 cm) *Hyles lineata* moths were abundant visitors and effective pollinators just after flowers opened (Fig. 1E), whereas fewer individuals of *Manduca quinquemaculata* visited these flowers later in the evening and no crepuscular bees were observed. Gregory (1964), who pioneered the use of paired control and emasculated flowers to determine the rate of outcrossed pollen deposition, found that most stigmas of *O. flava* subsp. *taraxacoides* received non-self pollen within the first 45 minutes after sunset. Similarly, studies of three different populations of *O. macrocarpa*, a related species with flowers whose floral tubes range from 9.2–12.3 cm in length, indicate that this species is pollinated effectively by *H. lineata*, *Dolba hyloeus* Drury, and other short-tongued (3–5 cm) hawkmoths (Nonnenmacher, 1999; Mothershead & Marquis, 2000; Moody-Weis & Heywood, 2001).

If flowers of *Oenothera flava* subsp. *taraxacoides* are effectively pollinated by short-tongued hawkmoths, why are their nectar tubes so spectacularly long? One hypothesis concerns the plants' acaulescent, rosette growth form combined with the moist, grassy meadows in which *O. flava* and its relatives often are found (Munz, 1930; Wagner, 1981). Hawkmoths generally prefer individual flowers that are highest above the ground (Herrera, 1993; Mothershead & Marquis, 2000), perhaps reflecting a predator avoidance strategy (Wasserthal, 1993; Dukas, 2001). In the absence of a bolting stem or inflorescence, directional selection for hypanthium length in *O. flava* subsp. *taraxacoides* may result in the projection of the floral visual display and/or landing platform higher above the substrate, as was suggested for *Nierembergia* Ruiz & Pav. by Cocucci (1991). A similar function is thought to be served by the sterile stipe of flowers in a related lineage, *Camissonia* Link sect. *Tetrapteron* (Munz) P. H. Raven, which also grow in wet, grassy habitats (Raven, 1969). An alternative hypothesis is that increased floral tube length of high-elevation *O. flava* ecotypes is a consequence of tight genetic correlation between this trait and some other character (e.g., leaf length), as was suggested for *O. caespitosa* subsp. *marginata* Munz by Wagner et al. (1985). Furthermore, it is possible that extreme tube length somehow enhances the interplay between short- and long-tongued hawkmoth visitors in ways that optimize the plants' male and/or female fitness. Direct manipulative experiments will be required to test these hypotheses, and additional research is needed to better understand the relative importance of autogamy and outcrossing in the North American species of *Oenothera* section *Lavauxia*.

Conclusions

Our comparative study provides conclusive answers to the four questions posed in the introduction. First, flower size, fragrance intensity and complexity, and nectar volume are reduced to some extent in the small-flowered, putatively autogamous taxa of *Oenothera* section *Lavauxia*, whereas flower color, scent, and nectar composition are comparable among all taxa. Thus, reduced floral advertisement and reward in autogamous taxa is a quantitative, rather than qualitative phenomenon. Second, the floral scent of *O. flava* subsp. *flava* is more similar to that of large-flowered *O. flava* subsp. *taraxacoides* than to the other putatively autogamous taxon, *O. triloba*, suggesting that phylogenetic affinity is more responsible than mating system for chemical composition in small-flowered section *Lavauxia* taxa. Third, the flowers of *O. acutissima* are distinct from those of *O. flava* subsp. *taraxacoides* in all measured characteristics, whereas the two isolated populations of *O. flava* subsp.

taraxacoides produce flowers that are similar in floral dimensions and scent chemistry but differ in nectar volume, floral mass, and odor intensity. These results suggest the intriguing hypothesis of independent derivation of large-flowered, high-elevation forms of *O. flava*. The remarkable variation in floral phenotypes within *O. flava* provides an unusual opportunity to study the genetic architecture and physiological costs of resource allocation to pollinator attraction through scent production and visual display. Finally, flowers of the narrow endemic *O. acutissima* were visited abundantly by the same spectrum of medium- to long-tongued hawkmoths that pollinate other night-blooming *Oenothera* throughout western North America. Additional field studies will be required to determine the relative contributions of self-pollination and outcrossing to the reproductive ecology of all section *Lavauxia* species.

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Appendix 1. Vouchers for *Oenothera* sect. *Lavauxia* examined in this study. All vouchers were deposited at ARIZ, US, and USCH. *RAR[R. A. Raguso]01-65* and *RAR99-46* were studied at the type localities for their respective taxa.

Oenothera acutissima W. L. Wagner. U.S.A. **Utah:** Daggett Co., Flaming Gorge Natl. Rec. Area, Greendale Campgr. & Hwy. 260, 1880 m, *RAR01-65* (ARIZ, US, USCH).

Oenothera flava subsp. *flava* (A. Nels.) Garrett. U.S.A. **Wyoming:** Teton Co., Teton National Forest, Hwy. 89, 1924 m, *RAR01-63* (ARIZ, US, USCH).

Oenothera flava subsp. *taraxacoides* (Woot. & Standl.) W. L. Wagner. U.S.A. **Arizona:** Navajo Co., mile marker 357 on Rte. 260, near Pine Top, 2350 m, *RAR98-146* (ARIZ, US, USCH).

Oenothera flava subsp. *taraxacoides* (Woot. & Standl.) W. L. Wagner. U.S.A. **New Mexico:** Otero Co., Rte. 82-Rte. 244 junction, 1.4 miles SE of Cloudcroft, 2800 m, *RAR99-46* (ARIZ, US, USCH).

Oenothera triloba Nutt. U.S.A. **Texas:** Bexar Co., Trinity University campus, San Antonio, 246 m, *RAR02-01* (collected by Donald Miller III) (ARIZ, US, USCH).

See Appendix 2, next page.

Appendix 2. Volatile compounds emitted by *Oenothera* section *Lavauxia* taxa. Numbers represent mean percentage (out of 100%) ± SE of total scent emitted. The data in the first column were collected in the field from the type locality of *O. acutissima*; all other data were collected in a common greenhouse environment. Compounds marked with * were identified by co-chromatography with known standards, those with ** using essential oils or natural products for which published GC MS data are available. For remaining compounds, putative names are provided when MS showed > 90% identity with entries from mass spectral libraries. MS of unidentified compounds are given in descending order of mass per unit charge (m/z), with % abundance relative to the base peak (100) in parentheses. AZ, Arizona populations of *O. flava* subsp. *taraxacoides*; NM, New Mexico populations of *O. flava* subsp. *taraxacoides*; nr., suggests that the compound shares a structural affinity to cis-cinerone but is not verifiably that compound; RT, retention time on carbowax GC column, in minutes; SPME, solid-phase microextraction. M + ? indicates a potential molecular ion for the unknown mass spectrum.

Scent Compound	RT	*O. acutissima* field	*O. acutissima*	*O. triloba*	*O. flava* subsp. *flava*	*O. flava* subsp. *taraxacoides* AZ	*O. flava* subsp. *taraxacoides* NM
Monoterpenes (7)							
α-pinene*	2.76			0.26 ± 0.16			0.12 ± 0.02
β-pinene*	4.08			0.33 ± 0.18			
sabinene*	4.31			0.04 ± 0.04	0.50 + 0.46		0.04 ± 0.01
β-myrcene*	5.07	0.25 ± 0.07	0.38 ± 0.11	0.38 ± 0.08			0.18 ± 0.02
Z-β-ocimene*	6.36	0.49 ± 0.13	0.85 ± 0.25	0.94 ± 0.20	1.37 ± 1.18	1.44 ± 0.85	0.47 ± 0.06
E-β-ocimene*	6.67	41.24 ± 10.48	48.96 ± 13.13	66.40 ± 8.50	3.38 ± 2.01	0.07 ± 0.03	31.14 + 3.36
1,3,8 p-mentha-triene	9.93		0.01 ± 0.01				
Oxygenated Monoterpenoids (9)							
1,8-cineole*	5.69			0.70 ± 0.70			
furanoid linalool oxide*	9.82	0.005 ± 0.003	0.01 ± 0.01			0.01 ± 0.01	
furanoid linalool oxide*	10.22	0.003 ± 0.002	0.02 ± 0.01			0.004 ± 0.004	
linalool*	11.19	15.39 ± 7.54	11.51 ± 8.76	2.43 ± 2.43		0.10 ± 0.04	
linalool epoxide	11.52	0.17 ± 0.10					
δ-terpineol	12.81			0.29 ± 0.29			
α-terpineol*	13.11			0.10 ± 0.10			
2,6-dimethyl-3,7-octadien-2,6-diol	15.90	0.01 ± 0.005					
2,6-dimethyl-1,7-octadien-3,6-diol	17.70	0.004 ± 0.002					
Unidentified terpene-like compounds (5)							
137(M + ?), 109(5), 95(8), 83(38), 69(24), 67(65), 55(33), 53(18), 43(92), 41(100)	8.59		0.03 ± 0.01				
137(M + ?), 123, 109(2), 96(6), 95(11), 83(38), 82(22), 69(30), 67(67), 55(34), 53(20), 43(31), 41(100)	9.17		0.20 ± 0.12				
nr. *Z*-cinerone	11.45		0.02 ± 0.01				
nr. *E*-cinerone	12.02		0.03 ± 0.02				
nr. isophytol	19.11	0.01 ± 0.01	0.04 + 0.04	6.77 ± 2.62	0.23 ± 0.19	0.58 + 0.15	0.08 ± 0.02
Sesquiterpenoids(4)							
E-β-caryophyllene*	12.04	0.03 ± 0.02	0.51 ± 0.34				

Appendix 2. Continued.

Scent Compound	RT	*O. acutissima* field	*O. acutissima*	*O. triloba*	*O. flava* subsp. *flava*	*O. flava* subsp. *taraxacoides* AZ	*O. flava* subsp. *taraxacoides* NM
α-humulene*	12.93		0.06 ± 0.04				
caryophyllene oxide*	16.43		0.10 ± 0.10				
E,E-farnesol	19.75		0.01 ± 0.01				
Aromatic Esters (2)							
methyl benzoate*	12.27	0.01 ± 0.007	0.02 ± 0.008	0.53 ± 0.32	In SPME only	0.18 ± 0.16	In SPME only
amyl benzoate*	15.61	0.01 ± 0.002	0.01 ± 0.004			0.02 ± 0.008	0.02 ± 0.004
Aliphatic compounds (9)							
isoamyl alcohol	6.12	0.12 ± 0.03	0.02 ± 0.01		0.72 ± 0.15	0.87 ± 0.15	0.41 ± 0.06
isoamyl butanoate	7.19	0.003 ± 0.002				0.17 ± 0.05	0.04 ± 0.008
isoamyl pentanoate	7.66	0.04 ± 0.01	0.06 ± 0.03			0.13 ± 0.04	0.24 ± 0.07
Z-3-hexenyl acetate*	7.91	0.08 ± 0.05	0.02 ± 0.02	7.58 ± 3.99			
Z-3-hexen-1-ol*	9.07	0.02 ± 0.01	0.01 ± 0.01				
isoamyl hexanoate	10.07					0.02 ± 0.01	<0.005
111(43), 97(40), 84(49), 83(57), 82(39), 69(92), 57(36), 55(92), 43(75), 41(100)	13.00	0.006 ± 0.005					
1-hexadecene	19.62			2.12 ± 1.79		0.05+0.04	0.19
octadecene	21.34			1.72 ± 1.24			
Nitrogenous Compounds (16)							
1-pyrroline	2.43	In SPME only	In SPME only	In SPME only	In SPME only	In SPME only	In SPME only
2-methylbutylnitrile	4.08	0.02 ± 0.01	0.20 ± 0.12			1.82 ± 0.51	0.52 ± 0.12
3-methylbutylnitrile	4.86	1.02 ± 0.12	2.01 ± 0.66		1.82 ± 0.72	5.10 ± 0.83	5.87 ± 0.84
nitro-2-methylpropane	6.52	0.005 ± 0.002	0.01 ± 0.007			0.06 ± 0.03	
4-methylpentylnitrile	6.73	0.05 ± 0.02	0.05 ± 0.03			0.03 ± 0.01	
nitro-2-methylbutane	8.07	0.03 ± 0.01	0.12 ± 0.05			0.95 ± 0.27	0.09 ± 0.02
nitro-3-methylbutane**	8.24	0.59 ± 0.06	0.74 ± 0.04		2.96 ± 1.28	0.94 ± 0.23	0.67 ± 0.08
2-methylpropanaldoxime	9.07	0.19 ± 0.08	0.08 ± 0.004			0.15 ± 0.04	0.14 ± 0.04
2-methylpropanaldoxime	9.31	0.09 ± 0.04	0.03 ± 0.01			0.03 ± 0.01	0.05 ± 0.01
2-methylbutyloxime*	10.48	6.10 ± 1.28	5.15 ± 1.62	1.48 ± 1.55	5.55 ± 1.24	36.16 ± 4.81	12.37 ± 1.51
3-methylbutyloxime*	10.56	17.81 ± 1.35	15.88 ± 4.65	3.14 ± 2.35	47.56 ± 2.27	27.14 ± 3.39	25.43 ± 1.93
2-methylbutyloxime*	10.74	2.16 ± 0.28	2.03 ± 0.51	0.71 ± 0.71	3.39 ± 0.65	8.23 ± 0.85	4.46 ± 0.54
3-methylbutyloxime*	11.03	13.11 ± 1.20	10.35 ± 2.93	2.97 ± 2.36	32.25 ± 1.49	14.06 ± 2.39	17.42 ± 2.13
methyl nicotinate*	14.09	0.92 ± 0.14	0.44 ± 0.13	1.02 ± 1.02	<0.005	1.65 ± 0.25	0.29 ± 0.06
phenylacetonitrile*	15.78	0.02 ± 0.01	0.03 ± 0.01		0.28 ± 0.12	0.06 ± 0.02	0.04 ± 0.01
nitro-2-phenylethane	17.80	0.003 ± 0.002					

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ADVANCES IN APOCYNACEAE: THE ENLIGHTENMENT, AN INTRODUCTION[1]

Mary E. Endress,[2] *Sigrid Liede-Schumann,*[3] *and Ulrich Meve*[3]

ABSTRACT

This issue of the *Annals of the Missouri Botanical Garden* is devoted to advances in the Apocynaceae s.l. and is based on the symposium, "Recent Progress in the Systematics of Apocynaceae," held at the XVII International Botanical Congress in Vienna in 2005. The collection of papers presented here spans the phylogenetic and geographic breadth of the family and includes at least one study focused on representatives from each of the five subfamilies: Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae. The papers range from higher-level phylogenetic analyses to more narrowly defined case studies and include new results in phylogenetics, taxonomy, biogeography, pollination biology, and a pharmacophagous plant–butterfly interaction involving pyrrolizidine alkaloids, as well as a new hypothesis for the evolution of pollinia and loss of a compitum in some advanced taxa. An updated classification scheme of the Apocynaceae is presented, with one additional tribe recognized in Rauvolfioideae (the Aspidospermeae) and three in Apocynoideae (the Nerieae and Odontadenieae as well as the Baisseeae, which are elevated in rank here). In Asclepiadoideae, seven subtribes are recognized within Asclepiadeae (the Asclepiadinae, Cynanchinae, Tylophorinae, Metastelmatinae, Orthosiinae, Oxypetalinae, and Gonolobinae) and four within Ceropegieae (the Anisotominae, Leptadeniinae, Heterostemminae, and Stapeliinae). Taken together, the papers here present the most up-to-date overview of the family available at this time.

Key words: Apocynaceae s.l., Apocynoideae, Asclepiadoideae, classification, monophyly, paraphyly, Periplocoideae, pharmacophagy, phylogeny, pollination biology, pollinia, pyrrolizidine alkaloids, Rauvolfioideae, Secamonoideae, systematics.

The issue you hold in your hands is the second Apocynaceae symposium issue and represents papers and posters presented in the symposium, "Recent Progress in the Systematics of Apocynaceae," held at the XVII International Botanical Congress in Vienna, which took place in July 2005. The 13 papers span the breadth of the Apocynaceae s.l., with contributions on all subfamilies and including new results in phylogenetic systematics, taxonomy, biogeography, pollination biology, and a pharmacophagous plant–animal mutualism between a species of *Prestonia* R. Br. (Echiteae) containing pyrrolizidine alkaloids and the lepidoptera that utilize them, as well as a new hypothesis for the evolution of pollinia and loss of

[1] We wish to thank all coauthors for their contribution, as well as for their hard work, patience, and cooperation in seeing through to completion this symposium issue of the *Annals of the Missouri Botanical Garden*. We also thank Victoria Hollowell, Beth Parada, and Diana Gunter of the Missouri Botanical Garden Press for their support and critical eye and for going the extra mile. Last, but certainly not least, we express our gratitude for the time and effort of the many reviewers, whose suggestions and critiques played a major role in the refinement of every paper, significantly improving this symposium issue.

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a compitum in some advanced taxa. Phylogenetic methods, especially those based on molecular data, indicate the presence of rampant parallel evolution throughout the family, involving numerous morphological characters, many of which were previously used as key characters in delimiting higher-level taxa (e.g., syncarpy vs. apocarpy, and fruit and seed type in Rauvolfioideae; corona structure in Asclepiadoideae) and some of which were once considered to be such complex characters that it was taken for granted that they could only have arisen once (e.g., pollinia). It is the most up-to-date compilation of papers currently available on Apocynaceae and is testimony to the significant advances since the first symposium volume.

The first symposium volume included seven papers published in volume 88 of the *Annals of the Missouri Botanical Garden* in 2001 and was based on talks presented in the symposium, "Evolution and Phylogenetics of the Apocynaceae s.l.," at the XVI International Botanical Congress held in St. Louis, Missouri, in 1999. Therein, the backbone of a consensus classification for the family was presented that included five subfamilies and 18 tribes (Endress & Stevens, 2001), and the broad acceptance of the reunification of Apocynaceae and Asclepiadaceae was announced for the first time since they were sundered by Robert Brown in 1810. Thus its title: The Renaissance of the Apocynaceae s.l.: Recent Advances in Systematics, Phylogeny, and Evolution.

In the six years since the publication of the first Apocynaceae symposium volume, Apocynaceae systematics has seen unabated activity, particularly within the largest subfamily, Asclepiadoideae. A number of phylogenetic studies focused on Asclepiadeae, the largest tribe of Asclepiadoideae, have brought a wealth of new information, allowing a much better understanding of the intertribal relationships. Two major clades have now emerged: the predominantly Old World ACT clade (including the Asclepiadinae Endl. ex Meisn., Cynanchinae K. Schum., and Tylophorinae (K. Schum.) Liede) and the exclusively New World MOG clade (including Metastelmatinae Endl. ex Meisn., Oxypetalinae K. Schum., and Gonolobinae (G. Don) Liede) (Liede & Täuber, 2000, 2002; Liede et al., 2002a, b; Rapini et al., 2003). More recently an additional subtribe, Orthosiinae Liede & Rapini, has been recognized within the MOG clade (Rapini et al., 2004; Liede-Schumann et al., 2005). Much progress has also been made in understanding relationships in the Old World tribe, Ceropegieae Decne. ex Orb. (Meve & Liede, 2001, 2002a, b), with its division into the four subtribes Anisotominae Meve & Liede, Leptadeniinae Meve & Liede, Heterostemminae Meve & Liede, and Stapeliinae G. Don (Meve & Liede, 2004). An interactive key to the genera of Periplocoideae, Secamonoideae, and Asclepiadoideae, as presently delimited, has been made accessible on the Internet, together with descriptions of all genera and, as far as are available, illustrations (Liede-Schumann & Meve, 2006, <http://www.uni-bayreuth.de/departments/planta2/research/databases/delta_as/index.htm>).

In the more basal subfamilies Rauvolfioideae and Apocynoideae, in which the differences between genera are much greater than in the Asclepiadoideae, progress has been made mainly in improving tribal circumscriptions (Simões et al., 2004, 2006, for Mesechiteae; Endress et al., 2007, for Alyxieae and Vinceae).

Since the previous volume represented the Renaissance of the Apocynaceae, it seems only logical to think of this volume as the Enlightenment. Herein the fetters of traditional circumscriptions of taxa, especially at the generic, tribal, or subtribal level, are cast aside and new boundaries are delimited.

The symposium issue opens with a broad phylogenetic study of the Rauvolfioideae, the plesiomorphic, basalmost subfamily of the Apocynaceae. Its diverse array of flower and fruit types has made it difficult to ascertain generic and tribal relationships in the past (Endress et al., 2007). Simões et al. (2007) phylogenetically analyze 41 genera of Rauvolfioideae, including representatives from all nine tribes, using a combination of morphology and five regions of the chloroplast genome. The subfamily forms a paraphyletic grade to the rest of the family, with its taxa dispersed among 12 clades. Syncarpous ovaries, indehiscent fruits, winged seeds, and simple styleheads, all of which have played an important role in previous tribal delimitation, appear to have evolved in parallel numerous times. Thus, tribes in earlier classifications (Pichon, 1948, 1949, 1950b; Leeuwenberg, 1994) are almost all polyphyletic. Of the nine tribes of Rauvolfioideae recognized by Endress and Bruyns (2000), six were found to be monophyletic, whereas three were paraphyletic. Despite its paraphyly, Rauvolfioideae is maintained for the present. A revised classification is presented, in which the constituent genera are realigned into 10 tribes.

Next, by Alvarado-Cárdenas and Ochoterena (2007), is a morphological phylogenetic analysis of the *Cascabela–Thevetia* complex, utilizing 55 morphological characters, many employed here for the first time. The genera *Cascabela* Raf., *Cerbera* L., and *Thevetia* L. are similar morphologically, as is reflected by their convoluted taxonomic history, which also involves *Ahouai* Tourn. and *Plumeriopsis* Rusby & Woodson (Woodson, 1937; Gensel, 1969; Lippold, 1980; Williams, 1996). The detailed phylogenetic

analysis presented includes all currently recognized species of *Cascabela* and *Thevetia*, as well as at least one species of each genus of the Plumerieae, sensu Endress and Bruyns (2000). The results indicate that *Cascabela* and *Thevetia* form the crown clade of the Plumerieae. Because there are several easy-to-diagnose characters to distinguish between them, the authors argue for the recognition of both genera. Another important result of this paper is the insight it provides for the usefulness of less frequently used morphological characters.

Livshultz et al. (2007) present a phylogenetic analysis of the Apocynoideae, using four chloroplast DNA (cpDNA) regions and 16 morphological characters, in the most comprehensive analysis of the Apocynoideae to date, with representatives of 59 of the 77 genera now in the subfamily. Here, many of the polytomies or weakly supported branches of the tree in Potgieter and Albert (2001) are resolved with strong support, providing the long-awaited insight into relationships among genera. The Apocynoideae and tribes therein are non-monophyletic as delimited in Pichon (1950a), Leeuwenberg (1994), and Endress and Bruyns (2000) as amended by Simões et al. (2004). Instead, the authors find biogeography to be an accurate predictor of phylogeny. The non-monophyly of the traditional Asclepiadaceae and the sister group relationship of the three African genera of the Baisseeae (Pichon ex De Kruif) M. E. Endress (Apocynoideae; the new combination is made in Appendix 1) to Secamonoideae–Asclepiadoideae is strongly supported for the first time and corroborated by two other contributions to this volume (Simões et al., 2007; Lahaye et al., 2007). Pollen aggregation and mass transfer are reconstructed as having evolved in parallel multiple times within the Apocynoideae–Periplocoideae–Secamonoideae–Asclepiadoideae (APSA) clade, in agreement with the results of Ionta and Judd (2007) for Periplocoideae, presented in this issue. This major study of Apocynoideae is the complement to Simões et al. (2007) for Rauvolfioideae, which, together, treat all tribes of the Apocynaceae s. str.

The contribution of Ionta and Judd (2007) represents the first large-scale phylogenetic investigation of the Periplocoideae using ribosomal and plastid DNA. Periplocoideae are restricted to the Old World and are unique among the five subfamilies of Apocynaceae in that they include taxa with and without pollinia (Verhoeven & Venter, 2001). In this study, all but three of the 33 currently recognized genera of Periplocoideae were included. The affinities of many genera are illuminated, and the previous tribal circumscriptions of Venter and Verhoeven (1997) are not supported. The relationship of Periplocoideae to Apocynoideae and the Secamonoideae–Asclepiadoideae clade, however, remains inconclusive. One of the most interesting results of this study is that pollinia in Periplocoideae are not only likely non-homologous with those in Secamonoideae–Asclepiadoideae, but that they have evolved at least three times in parallel within Periplocoideae.

Lahaye et al. (2007) present a molecular phylogeny of Madagascan Secamonoideae, which like Periplocoideae, are restricted to the Old World. The monophyly of *Pervillaea* Decne. and *Secamonopsis* Jum. is supported, whereas that of *Secamone* R. Br., by far the largest genus of the subfamily, is uncertain. The results suggest a recent and rapid diversification of Secamonoideae in Madagascar, with an explosive increase in morphological diversity. An unexpected bonus of the study is that the three African genera *Baissea* A. DC., *Motandra* A. DC., and *Oncinotis* Benth. of the Baisseeae are strongly supported as sister to the Secamonoideae–Asclepiadoideae clade, supporting results of Sennblad et al. (1998), Potgieter and Albert (2001), Livshultz et al. (2007), and Simões et al. (2007).

Meve and Liede-Schumann (2007) use chloroplast and nuclear DNA sequence data to investigate relationships within *Ceropegia* L., which, with 180 recognized species, is the largest genus of the Old World tribe Ceropegieae. The 36 analyzed species are dispersed in a grade composed of seven clades and are twice paraphyletic. The first paraphyly includes 11 species of *Ceropegia* together with all eight *Brachystelma* Sims exemplars. For the second paraphyly, the six endemic Madagascan *Ceropegia* species investigated and the East African species *C. robynsiana* Werderm. form an unresolved clade together with the 10 included stapeliads. Because no morphological, anatomical, or karyological characters are known at present to differentiate between these genera, the authors argue for a continued recognition of a paraphyletic *Ceropegia* for the time being.

Rapini et al. (2007) provide a biogeographic study of the New World Asclepiadoideae employing Bayesian inference and molecular dating on data from floristic inventories and 216 cpDNA sequences. In this analysis, the Asclepiadoideae are reconstructed as having arisen somewhere in the Old World 40 million years ago (Ma), confirming earlier hypotheses of an African origin for the subfamily (Macfarlane, 1933; Good, 1956; Meve & Liede, 2002b), fitting nicely with the sister relationship between the Secamonoideae–Asclepiadoideae clade and the African Baisseinae (Apocynoideae) reported here by Lahaye et al. (2007) and Livshultz et al. (2007). Four independent dispersal events to the New World are hypothesized: (1) the MOG clade (Metastelmatinae, Oxypetalinae, and Gonolobinae) colonized South

America via transoceanic long-distance dispersal from Africa 32 Ma; (2) *Cynanchum* L. subg. *Mellichampia* (A. Gray) Woodson (Cynanchinae) arrived in the New World 24 Ma; (3) *Asclepias* L. (Asclepiadinae) reached North America via the Bering Strait 20 Ma; and (4) *Marsdenia* R. Br. probably arrived in the New World 16 Ma.

In closer focus, Goyder et al. (2007) use nuclear and cpDNA to infer phylogenetic relationships among 19 of the 24 currently recognized genera in subtribe Asclepiadinae, particularly among the predominantly non-twining herbaceous genera around *Asclepias*. The subtribe is strongly supported as monophyletic, but generic delimitations, which have long been a source of contention (e.g., Brown, 1902–1903; Bullock, 1952; Goyder, 1995, 1998, 2001a, b), need to be revised. Either *Asclepias* should be expanded to encompass the entire subtribe, or it should be restricted to the New World and the generic limits of the Old World members should be redefined. The traditional floral characters that have been used to differentiate genera were found to be unreliable due to parallelism; vegetative features could prove to be more useful in this subtribe.

Oxypetalum R. Br. is one of the most characteristic and species-rich genera of Asclepiadoideae in southeastern Brazil. It is difficult to go into the field in this region without seeing at least one species of this genus. *Oxypetalum* belongs to the tribe Asclepiadeae, where recent phylogenetic studies place it in the Oxypetalinae core group (Liede-Schumann et al., 2005), the crown clade of the large and diverse MOG clade (Rapini et al., 2003, 2006). Marquete et al. (2007) revise the genus in Rio de Janeiro State, Brazil. Twenty-one species are recognized, of which four are endemic. A key to the species is included, and eight species are beautifully illustrated.

The ability of ithomiine and danaine butterflies to sequester pyrrolizidine alkaloids (PAs) and to use them for their own benefit is a well-known and fascinating phenomenon (e.g., Boppré, 1986). Here, Brehm et al. (2007) report the presence of PAs, including two novel retronecine monoesters, for the first time in *Prestonia amabilis* J. F. Morales (Apocynoideae, Echiteae) and their uptake by adults of eight species belonging to two groups of Lepidoptera: Ithomiinae butterflies (Nymphalidae) and Arctiinae moths (Arctiidae). It is postulated that *Prestonia* ancestors may have served as an ancestral source of PAs in the evolution of pharmacophagous behavior in the Ithomiinae butterflies. The presence of PAs has been documented for some species of *Parsonsia* R. Br., also in Echiteae (Edgar, 1984; Trigo et al., 1996). Another paper in this volume (Livshultz et al., 2007) documents phylogenetically for the first time the sister relationship of *Parsonsia* and *Prestonia*, despite the fact that the former genus is restricted to Asia and Australia and the latter to the Neotropics.

Postzygotic self-incompatibility has been demonstrated in some species of the evolutionarily advanced taxa of the APSA clade (Wyatt et al., 1998; Lipow & Wyatt, 1999, 2000), in which mixed loads of self- and cross-pollen could cause abortion of whole fruits and thus extreme waste of both cross-pollen and cross-fertilized ovules. Here, Wyatt and Lipow (2007) hypothesize that the evolution of pollinia and loss of a compitum in Apocynaceae s.l. are adaptations to prevent or compensate for the wasteful effects of mixed pollen loads. Having pollen massed together into pollinia provides that all of the pollen delivered to the stigmatic chambers during a single pollination event will be either compatible or incompatible. A compatible pollination will result in follicles with a full complement of cross-pollinated seeds (Broyles & Wyatt, 1990). Loss of the compitum enables one carpel to produce a follicle, even if the other one aborts from a mixed load of pollen.

Moré et al. (2007) report on the pollination biology of three night-flowering species of *Mandevilla* Lindl. native to Argentina, which are pollinated exclusively by long-tongued hawkmoths. They found that the two species with the longest corolla tubes (*M. longiflora* (Desf.) Pichon and *M. petraea* (A. St.-Hil.) Pichon, both with corolla tubes greater than 10 cm long) are pollinated by long- to very-long-tongued hawkmoths. Thus in these two species, the pollinator spectrum is restricted by the extremely long and slender corolla tube, a common strategy in various plant families (Grant & Grant, 1983a, b; Nilsson, 1988; Endress, 1994; Proctor et al., 1996; Johnson & Steiner, 1997). Surprisingly, the third species of *Mandevilla* investigated, *M. laxa* (Ruiz & Pav.) Woodson, with much shorter corolla tubes about 4 cm long, was also found to be pollinated by species of long- to very-long-tongued hawkmoths, with proboscis lengths greatly exceeding that of the corolla tube. In this species, the limiting factor was not the length of the corolla tube, but the operative width of the pollination chambers, which were too narrow for short-tongued hawkmoths, which have wider proboscides. Thus, in *Mandevilla*, both corolla tube length and operative width play an important role in restricting access to the flowers.

Finally, because floral morphology may also influence pollinator behavior and pollen transfer among New World *Asclepias*, Theiss et al. (2007) use multiple measures to evaluate the efficiency and effectiveness of diverse generalist pollinators on three sympatric species: *A. syriaca* L., *A. incarnata* L., and *A. verticillata* L. Except for the fidelity of butterflies on *A. incarnata* and the prevalence and constancy of

honeybees on *A. syriaca*, native North American bumblebees, carpenter bees, and sphecid wasps rank highest in all measures of pollinator effectiveness and exhibit less potential for geitonogamous pollen transfer than introduced honeybees. Pollinator effectiveness also varies with plant species, as insects carry more pollinia of *A. incarnata* and *A. verticillata* than of *A. syriaca* but appear more likely to insert *A. syriaca* pollinia into subsequent flowers, based on vector pollen loads from 15 sites. Even so, the high variability and individualistic actions of pollinators highlight the need for further study of spatio-temporal variation in pollinator effectiveness.

Although we have made great strides in our understanding of the phylogeny and relationships of the Apocynaceae in recent years, there remain certain regions of the family that are still poorly known, and even in those regions that are better investigated, there are taxonomic gaps to fill. It has become increasingly clear that both Rauvolfioideae and Apocynoideae are paraphyletic. We need to address this and find the most feasible resolution. Within the Rauvolfioideae, we particularly need to rigorously study Willughbeieae to determine generic limits and intertribal relationships. In Vinceae, we still lack conclusive evidence as to whether *Neisosperma* Raf. should be maintained as a genus distinct from *Ochrosia* Juss., and the systematic position of *Amsonia* Walter, which has been excluded from Vinceae (Endress et al., 2007), remains uncertain. We need to confirm the taxonomic composition of Melodineae and sort out its relationship with Hunterieae. In Apocynoideae, the relatives of *Rhabdadenia* Müll. Arg. and *Galactophora* Woodson need to be better investigated. Generic limits in Echiteae cry out to be elucidated; presently some genera are more or less defined only by the absence of a single morphological characteristic (e.g., *Laubertia* A. DC. is distinguished from closely related genera by the lack of calycine colleters). This is unsatisfactory, and we need to study the relationships within this tribe in greater depth and to search out new morphological characters to convincingly circumscribe its component genera. Despite the ever-increasing number of genetic regions analyzed and a significant new phylogeny of the Periplocoideae, their placement within the APSA clade remains uncertain. Certain floral specializations in the Periplocoideae, such as the staminal feet (Endress & Bruyns, 2000) and translators, appear to be morphologically intermediate between Apocynoideae and Secamonoideae–Asclepiadoideae. Until the systematic position of the Periplocoideae is elucidated, however, any hypotheses as to their evolutionary position are without substance. In Secamonoideae, most of the species molecularly studied to date are Madagascan; we need to strive for a broader sampling. The relationship of the mainly Asian *Toxocarpus* Wight & Arn. to *Secamone* is undetermined, and solving this conundrum would be a logical target in this subfamily. In Asclepiadoideae, the Marsdenieae is the tribe most in need of careful study. The first steps have been made in delimiting the large genus *Hoya* R. Br. from other genera in the *Hoya* alliance (Wanntorp et al., 2006; Wanntorp & Forster, 2007). Similarly, it remains to be determined whether the many satellite genera split off from *Marsdenia* deserve taxonomic recognition. The systematic position of the two enigmatic genera *Eustegia* R. Br. and *Emicocarpus* K. Schum. & Schltr. within Asclepiadoideae remains unclear. In Asclepiadeae, the larger genera of the MOG clade in South America are still insufficiently delimited, and their species numbers are uncertain.

Appendix 1 is an updated classification scheme for the Apocynaceae s.l. that lists subfamilies, tribes, and subtribes, incorporating the results presented in this symposium issue. The additional tribes and subtribes not present in the outline presented by Endress and Stevens (2001) are preceded by an asterisk.

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APPENDIX 1. Revised classification scheme for the Apocynaceae. The additional tribes and subtribes not present in the outline presented by Endress and Stevens (2001) are preceded by an asterisk.

RAUVOLFIOIDEAE Kostel. TYPE: *Rauvolfia* L. (83 genera)

***Aspidospermeae** Miers. TYPE: *Aspidosperma* Mart. & Zucc. (6 genera)

Aspidosperma Mart. & Zucc., *Geissospermum* Allemão, *Haplophyton* A. DC., *Microplumeria* Baill., *Strempeliopsis* Benth., *Vallesia* Ruiz & Pav.

Alstonieae G. Don. TYPE: *Alstonia* R. Br. (2 genera)

Alstonia R. Br., *Dyera* Hook. f.

Vinceae Duby. TYPE: *Vinca* L. (9 genera)

Catharanthus G. Don, *Kamettia* Kostel., *Kopsia* Blume, *Laxoplumeria* Markgr., *Ochrosia* Juss., *Petchia* Livera, *Rauvolfia* L., *Tonduzia* Pittier, *Vinca* L.

Willughbeieae A. DC. TYPE: *Willughbeia* Roxb. (18 genera)

Ancylobotrys Pierre, *Bousigonia* Pierre, *Chamaeclitandra* (Stapf) Pichon, *Clitandra* Benth., *Couma* Aubl., *Cyclocotyla* Stapf, *Cylindropsis* Pierre, *Dictyophleba* Pierre, *Hancornia* Gomes, *Lacmellea* H. Karst., *Landolphia* P. Beauv., *Leuconotis* Jack, *Orthopichonia* H. Huber, *Pacouria* Aubl., *Parahancornia* Ducke, *Saba* (Pichon) Pichon, *Vahadenia* Stapf, *Willughbeia* Roxb.

Tabernaemontaneae G. Don. TYPE: *Tabernaemontana* L. (19 genera)

Ambelania Aubl., *Bonafousia* A. DC., *Callichilia* Stapf, *Calocrater* K. Schum., *Carvalhoa* K. Schum., *Crioceras* Pierre, *Macoubea* Aubl., *Molongum* Pichon, *Mucoa* Zarucchi, *Neocouma* Pierre, *Rhigospira* Miers, *Schizozygia* Baill., *Spongiosperma* Zarucchi, *Stemmadenia* Benth., *Stenosolen* (Müll. Arg.) Markgr., *Tabernaemontana* L., *Tabernanthe* Baill., *Voacanga* Thouars, *Woytkowskia* Woodson

Melodineae G. Don. TYPE: *Melodinus* J. R. Forst. & G. Forst. (4 genera)

Craspidospermum Bojer ex A. DC., *Melodinus* J. R. Forst. & G. Forst., *Pycnobotrya* Benth., *Stephanostegia* Baill.

Alyxieae G. Don. TYPE: *Alyxia* Banks ex R. Br. (7 genera)

Alyxia Banks ex R. Br., *Chilocarpus* Blume, *Condylocarpon* Desf., *Lepinia* Decne., *Lepiniopsis* Valeton, *Plectaneia* Thouars, *Pteralyxia* K. Schum.

Hunterieae Miers. TYPE: *Hunteria* Roxb. (4 genera)

Gonioma E. Mey., *Hunteria* Roxb., *Picralima* Pierre, *Pleiocarpa* Benth.

Plumerieae E. Mey. TYPE: *Plumeria* L. (10 genera)

Allamanda L., *Anechites* Griseb., *Cameraria* L., *Cerbera* L., *Cerberiopsis* Vieill. ex Pancher & Sébert, *Himatanthus* Willd. ex Schult., *Mortoniella* Woodson, *Plumeria* L., *Skytanthus* Meyen, *Thevetia* L.

Carisseae Dumort. TYPE: *Carissa* L. (2 genera)

Acokanthera G. Don, *Carissa* L.

Rauvolfioideae incertae sedis (2 genera)

Amsonia Walter, *Diplorhynchus* Welw. ex Ficalho & Hiern.

APOCYNOIDEAE Burnett. TYPE: *Apocynum* L. (80 genera)

Wrightieae G. Don. TYPE: *Wrightia* R. Br. (3 genera)
Pleioceras Baill., *Stephanostema* K. Schum., *Wrightia* R. Br.

***Nerieae** Baill. TYPE: *Nerium* L. (6 genera)
Adenium Roem. & Schult., *Alafia* Thouars, *Farquharia* Stapf, *Isonema* R. Br., *Nerium* L., *Strophanthus* DC.

Malouetieae Müll. Arg. TYPE: *Malouetia* A. DC. (11 genera)
Illovoodsonia Markgr., *Carruthersia* Seem., *Funtumia* Stapf, *Holarrhena* R. Br., *Kibatalia* G. Don, *Malouetia* A. DC., *Malouetiella* Pichon, *Mascarenhasia* A. DC., *Neobracea* Britton, *Pachypodium* Lindl., *Spirolobium* Baill.

Apocyneae Rchb. TYPE: *Apocynum* L. (23 genera)
Aganonerion Pierre ex Spire, *Aganosma* (Blume) G. Don, *Amalocalyx* Pierre, *Amphineurion* (A. DC.) Pichon, *Anodendron* A. DC., *Apocynum* L., *Baharuia* D. J. Middleton, *Beaumontia* Wall., *Chonemorpha* G. Don, *Cleghornia* Wight, *Dewevrella* De Wild., *Epigynum* Wight, *Ichnocarpus* R. Br., *Ixodonerium* Pit., *Micrechites* Miq., *Papuechites* Markgr., *Parameria* Benth., *Parepigynum* Tsiang & P. T. Li, *Pottsia* Hook. & Arn., *Sindechites* Oliv., *Trachelospermum* Lem., *Urceola* Roxb., *Vallaris* Burm. f.

***Odontadenieae** Miers. TYPE: *Odontadenia* Benth. (6 genera)
Cycladenia Benth., *Elytropus* Müll. Arg., *Odontadenia* Benth., *Secondatia* A. DC., *Stipecoma* Müll. Arg., *Thyrsanthella* (Baill.) Pichon

Mesechiteae Miers. TYPE: *Mesechites* Müll. Arg. (5 genera)
Illomarkgrafia Woodson, *Forsteronia* G. Mey., *Mandevilla* Lindl., *Mesechites* Müll. Arg., *Tintinnabularia* Woodson

Echiteae G. Don. TYPE: *Echites* P. Browne (20 genera)
Ingadenia Miers, *Artia* Guillaumin, *Asketanthera* Woodson, *Bahiella* J. F. Morales, *Echites* P. Browne, *Ecua* D. J. Middleton, *Fernaldia* Woodson, *Hylaea* J. F. Morales, *Laubertia* A. DC., *Macropharynx* Rusby, *Parsonsia* R. Br., *Peltastes* Woodson, *Pentalinon* Voigt, *Prestonia* R. Br., *Rhabdadenia* Müll. Arg., *Rhodocalyx* Müll. Arg., *Salpinctes* Woodson, *Temnadenia* Miers, *Thenardia* Kunth, *Thoreauea* J. K. Williams

***Baisseeae** (Pichon ex De Kruif) M. E. Endress, stat. nov. Baisseinae Pichon ex De Kruif, Meded. Landbouwhoogeschool Wageningen 83(7): 2. 1983. TYPE: *Baissea* A. DC. 1844 (3 genera)
Baissea A. DC., *Motandra* A. DC., *Oncinotis* Benth.

Apocynoideae incertae sedis (3 genera)
Eucorymbia Stapf, *Galactophora* Woodson, *Vallariopsis* Woodson

PERIPLOCOIDEAE R. Br. ex Endl. TYPE: *Periploca* L. (33 genera)
Atherandra Decne., *Baroniella* Costantin & Gallaud, *Baseonema* Schltr. & Rendle, *Batesanthus* N. E. Br., *Buckollia* Venter & R. L. Verh., *Camptocarpus* Decne., *Cryptolepis* R. Br., *Cryptostegia* R. Br., *Decalepis* Wight & Arn., *Ectadium* E. Mey., *Epistemma* D. V. Field & J. B. Hall, *Finlaysonia* Wall., *Gymnanthera* R. Br., *Hemidesmus* R. Br., *Kappia* Venter, A. P. Dold & R. L. Verh., *Ischnolepis* Jum. & H. Perrier, *Maclaudia* Venter & R. L. Verh., *Mondia* Skeels, *Myriopteron* Griff., *Omphalogonus* Baill., *Pentopetia* Decne., *Periploca* L., *Petopentia* Bullock, *Phyllanthera* Blume, *Raphionacme* Harv., *Sacleuxia* Baill., *Sarcorrhiza* Bullock, *Schlechterella* K. Schum., *Stomatostemma* N. E. Br., *Streptocaulon* Wight & Arn., *Tacazzea* Decne., *Telectadium* Baill, *Zygostelma* Benth.

SECAMONOIDEAE Endl. (8 genera)

Secamoneae G. Don. TYPE: *Secamone* R. Br.
Calyptranthera Klack., *Genianthus* Hook. f., *Goniostemma* Wight, *Perrillaea* Decne., *Secamone* R. Br., *Secamonopsis* Jum., *Toxocarpus* Wight & Arn., *Trichosandra* Decne.

ASCLEPIADOIDEAE R. Br. ex Burnett. TYPE: *Asclepias* L. (172 genera)

Fockeeae Kunze, Meve & Liede. TYPE: *Fockea* Endl. (2 genera)
Cibirhiza Bruyns, *Fockea* Endl.

Marsdenieae Benth. TYPE: *Marsdenia* R. Br. (27 genera)
Anatropanthus Schltr., *Anisopus* N. E. Br., *Asterostemma* Decne., *Campestigma* Pierre ex Costantin, *Cathetostemma* Blume, *Cionura* Griseb., *Clemensiella* Schltr., *Cosmostigma* Wight, *Dischidia* R. Br., *Dolichopetalum* Tsiang, *Gongronema* (Endl.) Decne., *Gunnessia* P. I. Forst., *Gymnema* R. Br., *Heynella* Backer, *Hoya* R. Br., *Jasminanthes* Blume, *Lygisma* Hook. f., *Marsdenia* R. Br., *Oreosparte* Schltr., *Pycnorhachis* Benth., *Rhyssolobium* E. Mey., *Sarcolobus* R. Br., *Stephanotis* Thouars, *Stigmatorhynchus* Schltr., *Telosma* Coville, *Treutlera* Hook. f., *Wattakaka* Hassk.

Ceropegieae Decne. ex Orb. TYPE: *Ceropegia* L. (46 genera)

***Anisotominae** Meve & Liede. TYPE: *Anisotoma* Fenzl (5 genera)
Anisotoma Fenzl, *Emplectanthus* N. E. Br., *Veoschumannia* Schltr., *Riocreuxia* Decne., *Sisyranthus* E. Mey.

***Heterostemminae** Meve & Liede. TYPE: *Heterostemma* Wight & Arn. (1 genus)
Heterostemma Wight & Arn.

***Leptadeniinae** Meve & Liede. TYPE: *Leptadenia* R. Br. (4 genera)
Conomitra Fenzl, *Leptadenia* R. Br., *Orthanthera* Wight, *Pentasachme* Wall. ex Wight

***Stapeliinae** G. Don. TYPE: *Stapelia* L. (35 genera)
Apteranthes J. C. Mikan, *Australluma* Plowes, *Baynesia* Bruyns, *Boucerosia* Wight & Arn., *Brachystelma* Sims, *Caralluma* R. Br., *Caudanthera* Plowes, *Ceropegia* L., *Desmidorchis* Ehrenb., *Duvalia* Haw., *Duvaliandra* M. G. Gilbert, *Echidnopsis* Hook. f., *Edithcolea* N. E. Br., *Hoodia* Sweet ex Decne., *Huernia* R. Br., *Larryleachia* Plowes, *Lavrania* Plowes, *Monolluma* Plowes, *Notechidnopsis* Lavranos & Bleck, *Ophionella* Bruyns, *Orbea* Haw., *Orbeanthus* L. C. Leach, *Pectinaria* Haw., *Piaranthus* R. Br., *Pseudolithos* P. R. O. Bally, *Quaqua* N. E. Br., *Rhytidocaulon* P. R.

O. Bally, *Richtersveldia* Meve & Liede, *Socotrella* Bruyns & A. G. Miller, *Stapelia* L., *Stapelianthus* Choux ex A. C. White & B. Sloane, *Stapeliopsis* Pillans, *Tavaresia* Welw., *Tridentea* Haw., *Tromotriche* Haw., *White-Sloanea* Chiov.

Asclepiadeae (R. Br.) Duby. TYPE: *Asclepias* L. (97 genera)

*__Astephaninae__ Endl. ex Meisn. TYPE: *Astephanus* R. Br. (3 genera)

Astephanus R. Br., *Microloma* R. Br., *Oncinema* Arn.

*__Asclepiadinae__ Endl. ex Meisn. TYPE: *Asclepias* L. (23 genera)

Aidomene Stopp, *Asclepias* L., *Aspidoglossum* E. Mey., *Aspidonepsis* Nicholas & Goyder, *Calotropis* R. Br., *Cordylogyne* E. Mey., *Fanninia* Harv., *Glossostelma* Schltr., *Gomphocarpus* R. Br., *Kanahia* R. Br., *Lagarinthus* E. Mey., *Margaretta* Oliv., *Miraglossum* Kupicha, *Odontostelma* Rendle, *Pachycarpus* E. Mey., *Parapodium* E. Mey., *Pergularia* L., *Schizoglossum* E. Mey., *Stathmostelma* K. Schum., *Stenostelma* Schltr., *Trachycalymma* Bullock, *Woodia* Schltr., *Xysmalobium* R. Br.

*__Tylophorinae__ (K. Schum.) Liede. TYPE: *Tylophora* R. Br. (9 genera)

Biondia Schltr., *Blyttia* Arn., *Diplostigma* K. Schum., *Goydera* Liede, *Pentatropis* R. Br., *Pleurostelma* Baill., *Rhyncharrhena* F. Muell., *Tylophora* R. Br., *Vincetoxicum* Wolf

*__Cynanchinae__ K. Schum. TYPE: *Cynanchum* L. (14 genera)

Adelostemma Hook. f., *Cynanchum* L., *Glossonema* Decne., *Graphistemma* (Champ. ex Benth.) Champ. ex Benth., *Holostemma* R. Br., *Mahawoa* Schltr., *Merrillanthus* Chun & Tsiang, *Metaplexis* R. Br., *Odontanthera* Wight, *Pentarrhinum* E. Mey., *Pentastelma* Tsiang & P. T. Li, *Raphistemma* Wall., *Schizostephanus* Hochst. ex Benth., *Sichuania* M. G. Gilbert & P. T. Li

*__Metastelmatinae__ Endl. ex Meisn. TYPE: *Metastelma* R. Br. (14 genera)

Barjonia Decne., *Blepharodon* Decne., *Ditassa* R. Br., *Hemipogon* Decne., *Hypolobus* E. Fourn., *Macroditassa* Malme, *Metastelma* R. Br., *Minaria* T. U. P. Konno & Rapini, *Nautonia* Decne., *Nephradenia* Decne., *Peplonia* Decne., *Petalostelma* E. Fourn., *Rhyssostelma* Decne., *Tassadia* Decne.

*__Orthosiinae__ Liede & Rapini. TYPE: *Orthosia* Decne. (3 genera)

Jobinia E. Fourn., *Orthosia* Decne., *Scyphostelma* Baill.

*__Oxypetalinae__ K. Schum. TYPE: *Oxypetalum* R. Br. (10 genera)

Amblyopetalum (Griseb.) Malme, *Araujia* Brot., *Funastrum* E. Fourn., *Morrenia* Lindl., *Oxypetalum* R. Br., *Philibertia* Kunth, *Schistogyne* Hook. & Arn., *Stenomeria* Turcz., *Tweedia* Hook. & Arn., *Widgrenia* Malme

*__Gonolobinae__ (G. Don) Liede. TYPE: *Gonolobus* Michx. (13 genera)

Dictyanthus Decne., *Fischeria* DC., *Gonolobus* Michx., *Gyrostelma* E. Fourn., *Macroscepis* Kunth, *Matelea* Aubl., *Pherotrichis* Decne., *Polystemma* Decne., *Prosthecidiscus* Donn. Sm., *Rojasia* Malme, *Schubertia* Mart., *Stelmagonum* Baill., *Trichosacme* Zucc.

Asclepiadeae incertae sedis (8 genera)

Calciphila Liede & Meve, *Diplolepis* R. Br., *Emicocarpus* K. Schum. & Schltr., *Eustegia* R. Br., *Oxystelma* R. Br., *Pentacyphus* Schltr., *Seshagiria* Ansari & Hemadri, *Solenostemma* Hayne

PHYLOGENY AND SYSTEMATICS OF THE RAUVOLFIOIDEAE (APOCYNACEAE) BASED ON MOLECULAR AND MORPHOLOGICAL EVIDENCE[1]

André O. Simões,[2,4] Tatyana Livshultz,[3] Elena Conti,[2] and Mary E. Endress[2]

ABSTRACT

To elucidate deeper relationships within Rauvolfioideae (Apocynaceae), a phylogenetic analysis was conducted using sequences from five DNA regions of the chloroplast genome (*matK*, *rbcL*, *rpl16* intron, *rps16* intron, and 3′ *trnK* intron), as well as morphology. Bayesian and parsimony analyses were performed on sequences from 50 taxa of Rauvolfioideae and 16 taxa from Apocynoideae. Neither subfamily is monophyletic, Rauvolfioideae because it is a grade and Apocynoideae because the subfamilies Periplocoideae, Secamonoideae, and Asclepiadoideae nest within it. In addition, three of the nine currently recognized tribes of Rauvolfioideae (Alstonieae, Melodineae, and Vinceae) are polyphyletic. We discuss morphological characters and identify pervasive homoplasy, particularly among fruit and seed characters previously used to delimit tribes in Rauvolfioideae, as the major source of incongruence between traditional classifications and our phylogenetic results. Based on our phylogeny, simple style-heads, syncarpous ovaries, indehiscent fruits, and winged seeds have evolved in parallel numerous times. A revised classification is offered for the subfamily, its tribes, and inclusive genera.

Key words: Apocynaceae, classification, homoplasy, molecular phylogenetics, morphology, Rauvolfioideae, systematics.

During the past decade, phylogenetic studies, especially those employing molecular data, have significantly improved our understanding of higher-level relationships within Apocynaceae s.l., leading to the recognition of this family as a strongly supported clade composed of the traditional Apocynaceae s. str. and Asclepiadaceae s.l. (Judd et al., 1994; Endress et al., 1996; Sennblad & Bremer, 1996, 2002; Potgieter & Albert, 2001). The greatest progress has been made within the speciose asclepiads, where we now have a fairly reliable backbone upon which to base a tribal and, in some cases even a subtribal, classification (Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Liede et al., 2002a, b; Rapini et al., 2003; Meve & Liede, 2002, 2004; Verhoeven et al., 2003; Liede & Meve, 2004; Liede-Schumann et al., 2005).

Despite significant insights gained from studies over the past 10 years, especially from the broad-scale studies based on *rbcL* by Sennblad and Bremer (1996, 2002) and the *trnL-F* intergenic spacer by Potgieter and Albert (2001), a number of uncertainties remain within the Apocynaceae s. str. In particular, the resolution of the deeper nodes in both Rauvolfioideae as well as Apocynoideae has proven to be

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problematic. More narrowly focused studies have concentrated for the most part on improving understanding of relationships in the Apocynoideae (Sennblad et al., 1998; Simões et al., 2004; Livshultz et al., 2007, this issue). In contrast, studies in the subfamily Rauvolfioideae have lagged behind. This is probably due in part to a lack of charisma in several genera having similar-looking small, inconspicuous, white, salverform flowers. Due to the relative dearth of taxonomically useful traits in the flowers, traditional classifications of Rauvolfioideae were based almost exclusively on fruit and seed characters (Schumann, 1895; Pichon, 1948a, b, 1949; Leeuwenberg, 1994a). Although the diverse array of fruit and seed types in Rauvolfioideae provides easy characters to develop a classification, preliminary molecular and morphological phylogenetic studies suggest that fruit and seed characters are homoplasious and that most higher taxa in previous classifications are not monophyletic (Potgieter & Albert, 2001).

In this study, we aim to elucidate the deeper relationships in Rauvolfioideae that remained unresolved or poorly supported in recent phylogenetic studies in order to evaluate the congruence between the retrieved monophyletic groups and the genera, as well as supergeneric taxa recognized in the most recent classification (Endress & Bruyns, 2000). Because denser taxon and character sampling is known to improve phylogenetic resolution (Graybeal, 1998; Rannala et al., 1998; Soltis et al., 1998, 2001), we expanded both for Rauvolfioideae compared to previous phylogenetic studies (Potgieter & Albert, 2001; Sennblad & Bremer, 2002), including a total of 76 taxa and characters from five plastid loci (*matK*, *rbcL*, *rpl16* intron, *rps16* intron, and 3′ *trnK* intron), as well as morphology. We compare the resulting phylogenetic hypotheses within Rauvolfioideae with the classifications of Leeuwenberg (1994a) and Endress and Bruyns (2000), discuss the morphological features traditionally used to define tribes that proved to be consistent with the retrieved clades, and propose conservative modifications to the subfamily's classification until better resolved and more strongly supported phylogenies become available. We then use this information as a basis to discuss character evolution in this subfamily and, finally, to provide improved circumscription at the tribal level.

Materials and Methods

Taxon Sampling

Fifty taxa (41 genera) of Rauvolfioideae and 16 genera of Apocynoideae were employed in this study, including representatives from all 14 tribes recognized in Apocynaceae s. str. by Endress and Bruyns (2000). In addition, two genera of Periplocoideae, one genus of Secamonoideae, and two genera of Asclepiadoideae were included. Outgroup taxa, *Gentiana* L. (Gentianaceae), *Gelsemium* Juss. (Gelsemiaceae), *Strychnos* L., and *Mitrasacme* Labill. (Loganiaceae), were chosen from other families of Gentianales to include potential close sister groups to Apocynaceae (Backlund et al., 2000). The taxa analyzed, voucher information, and GenBank accession numbers are listed in Appendix 1. Sequences for 12 genera and 37 species are reported here for the first time and are analyzed together with sequences generated in previous studies (Sennblad & Bremer, 1996, 2002; Endress et al., 1996; Sennblad et al., 1998; Potgieter & Albert, 2001; Simões et al., 2004; Livshultz et al., 2007).

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted from silica-dried leaf material or from herbarium specimens using DNeasy Plant Mini Kits (Qiagen, Valencia, California, U.S.A.) following the manufacturer's protocol. For some problematic taxa, particularly those samples taken from herbarium material, a modified CTAB methodology based on the standard CTAB DNA extraction method (Doyle & Doyle, 1987) but using proteinase K instead of ß-mercaptoethanol, proved to be a more efficient means of extraction. Five plastid loci, comprising three non-coding (*rpl16*, *rps16*, and 3′ *trnK* introns) and two coding (*matK* and *rbcL*) regions, were amplified. Double-stranded DNA was amplified by polymerase chain reaction (PCR) on a Biometra Tgradient machine (Biometra, Göttingen, Germany), applying a thermal cycling program consisting of 40 cycles of denaturation (95°C, 30 sec.), annealing (52°C, 1 min.), and chain extension (72°C, 90 sec.). The *trnK* intron and *matK* were co-amplified in a single PCR reaction, and the thermal cycling program was modified in the following steps: denaturation (94°C, 30 sec.) and annealing (58°C, 1 min.). Reactions were terminated with a final extension of 4 min. at 72°C for the *rpl16* and *rps16* introns, and 7 min. for the *trnK* intron, *matK*, and *rbcL* genes. All PCR reactions were performed in a total reaction volume of 25 µl, using 2.5 mM $MgCl_2$, 10× PCR* Buffer (Amersham Biosciences), 0.25 mM of dNTP, 0.5 units of Taq DNA polymerase (Amersham Biosciences, lot 17544), 1 to 4 µl of bovine serum albumin (Sigma, Steinheim, Germany), and 0.1 mM of each primer. Amplification as one piece was possible for most of the taxa. In those cases where no products were obtained by using primers external to the target regions, smaller fragments were amplified by using

a combination of external and internal primers. Most of the primers utilized in our analyses were obtained from previous studies, as follows: Zurawski et al. (1981) for the *rbcL* gene, Simões et al. (2004) for the *rpl16* and *rps16* introns, and Civeyrel and Rowe (2001) for the *trnK* intron and *matK* gene. As reported in Sennblad and Bremer (1996), primers rbcLz-234 and rbcLz-895 were modified for use in the *rbcL* gene, as follows: 5′-CGTTACAAAGGKCGATGCTAC-CAYATCGA-3′ for the former, and 5′-GCAGTTATT-GATAGACAGAARAATCATGG-3′ for the latter. The primers matK438F (5′-TTTKGKGTTAGATATAY-TAAT-3′) and matK756R (5′-TAGAAATAGAT-TAGTTCAAGA-3′) were designed by us for use in sequencing of the *matK* gene. Amplified PCR products were then purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences). No amplified products of the 3′ *trnK* intron were obtained for *Gelsemium sempervirens* Ait. and *Theretia peruviana* Merr.

Cycle-sequencing reactions were carried out using an ABI Prism Big Dye Terminator Cycle Sequencing Ready Extraction Kit (Perkin Elmer, Applied Biosystems, Applera Europe BV, Rotkreuz, Switzerland). Sequence products were purified on MicroSpin G-50 columns (Amersham Pharmacia Biotech Europe, Dübendorf, Switzerland) and loaded on an ABI Prism 377 DNA sequencer (Perkin Elmer). Complementary strands were edited and assembled with Sequencher 1.2 (Gene Codes, Ann Arbor, Michigan, U.S.A.).

DATA MATRIX COMPOSITION AND ALIGNMENT

Nucleotide sequences of the studied plastid loci were manually assembled for each taxon. Aligned sequence matrices of the individual regions are available from the first author upon request. Aligned gaps were manually coded as presence/absence characters applying the single indel coding method described by Simmons and Ochoterena (2000) for *matK*, and by using the software Gapcoder (Young & Healy, 2003) for the other loci. Manual confirmation of the coded gap characters showed that the Gapcoder software performed well even in cases of overlapping gaps with different start and/or ending positions, and no further adjustments in the matrices were necessary. All coded gaps were then added to the sequence matrix and used in further analyses.

Forty-one parsimony-informative morphological characters were coded and analyzed with maximum parsimony. For character coding, herbarium and fresh specimens, pickled flowers, and when available, flower sections provided by the last author were used. Information for some characters was taken from the literature (e.g., Woodson, 1928; Pichon, 1948a, b, 1949; Erdtman, 1952; Bisset, 1960, 1980, 1992; Hegnauer, 1970, 1989; Ornduff, 1970; Gadella, 1980; Leeuwenberg & Leenhouts, 1980; Barink, 1983; Coppen & Cobb, 1983; Kisakürek et al., 1983; Goldblatt, 1984, 1988; Nilsson, 1986; Chatterjee et al., 1987; Endress et al., 1990; Goldblatt & Johnson, 1990, 1991; Leeuwenberg, 1991; Middleton, 1993, 2003; Hartmann & Witte, 1995; Li et al., 1995a, b; Doll et al., 1996; Forster & Williams, 1996; Albers & Meve, 2001; van der Ham et al., 2001; Verhoeven & Venter, 2001; Humberto et al., 2004; Middleton et al., 2005). The morphological matrix, and the character and character states, including explanatory notes on characters, are given in Appendices 2 and 3.

PARSIMONY ANALYSIS

A total of six data sets were subjected to phylogenetic analysis, corresponding to the five loci sequenced plus morphology. Because simultaneous analysis of combined data has been proposed as the best approach to phylogenetic inference (Nixon & Carpenter, 1996), we tested the combinability of all partitions by searching for incongruence between individual data sets. For this, we compared the results on a node-to-node basis of all individual data sets with respect to levels of resolution and bootstrap support, as applied by other authors (e.g., Wiens, 1998; Sheahan & Chase, 2000; Whitten et al., 2000; Reeves et al., 2001).

Maximum parsimony (MP) analyses were performed using PAUP* 4.0b (Swofford, 2000). All characters were unordered and equally weighted. Polymorphisms in the data matrix were treated as such, rather than as uncertainties. A heuristic search for the most parsimonious trees included an initial round of tree searches with 1000 random addition sequence replicates (RASR), holding 10 trees at each step, tree bisection-reconnection (TBR) branch swapping with MULTREES and steepest descent in effect, saving a maximum of 50 trees at each replicate. All shortest trees retained in the memory were then included in a second round of searches involving exhaustive TBR branch swapping. Relative support for each node was estimated using the bootstrap resampling procedure (Felsenstein, 1985) as implemented in PAUP, employing a heuristic search with 500 replicates, 250 RASR, three trees held at each step, TBR branch swapping with steepest descent and MULTREES in effect, saving 10 trees at each RASR.

In order to identify the synapomorphies that are congruent with each of the major clades of Rauvolfioideae retrieved in our analyses, morphological characters were mapped onto one of the 28 most parsimonious trees resulting from the total evidence parsimony analysis, using MacClade 4.0 (Maddison &

Maddison, 2000). Unambiguous changes were then reconstructed with maximum parsimony, applying both accelerated (ACCTRAN) and delayed (DELTRAN) character state optimizations. Alternative optimizations were also reconstructed in five other most parsimonious trees to investigate the influence of alternative topologies on possible interpretations of character evolution.

BAYESIAN ANALYSIS

Bayesian inference was performed using Mr Bayes version 3.0 (Ronquist & Huelsenbeck, 2003). Data were partitioned into the categories: *rpl16* intron, *rps16* intron, 3′ *trnK* intron, *matK*, *rbcL*, and gaps. The most optimal model of sequence evolution for each partition was selected using Modeltest version 3.6 (Posada & Crandall, 1998). A combined data set of all partitions, including gaps, was analyzed, applying separate models to each data partition and with parameters estimated separately for each individual partition. Starting model parameters were assigned uniform prior probabilities and further estimated during the analysis, by allowing them to vary independently among data partitions. One million generations were run, with parameters sampled every 1000 generations. Based on the inspection of the likelihood scores for each generation, the first 250 sampled generations were considered as burn-in and thus discarded from subsequent analyses.

RESULTS

ANALYSES OF THE CHLOROPLAST DNA DATA SETS

Multiple sequence alignment was straightforward for *rbcL*, with no observed gaps, and for *matK*, required only a few gaps that, without exception, occurred in multiples of three. Alignment was also straightforward for the 3′ *trnK* intron and the *rps16* intron, but proved to be more difficult for the *rpl16* intron due to the large number of gaps and mononucleotide repeats. A total of 274 characters, including regions of mononucleotide repeats with variable length between taxa as well as composed by nested gaps resulting from ambiguous alignment, were excluded from the analysis.

Tree length, consistency index (CI), and retention index (RI) values for the cladograms that resulted from the maximum parsimony analyses of individual and combined data sets are summarized in Table 1. Among the individual molecular data sets, *matK* provided the best-resolved cladogram, with most of the nodes receiving bootstrap support (BS) higher than 50%. The highest proportion of parsimony-informative characters, as well as the highest CI and RI values,

Table 1. Summary of sequence length, variability, and parsimony-tree parameters for individual and combined data sets. Tree length, consistency index (CI), and retention index (RI) were calculated based on parsimony-informative characters only. Coded gaps, when present, were considered for calculations of the total number and percentage of parsimony-informative characters for both individual and combined data sets. NA = Data not applicable.

	matK gene	3′ half of *trnK* intron	*rpl16* intron	Molecular combined	Morphology
Aligned length	1683	366	1181	5563	41
Range of sequence length	1098–1164	190–247	886–1081	NA	NA
No. of coded gaps	48	79	194	490	NA
Total no. of parsimony-informative characters (% total no.)	528 (30.5%)	122 (27.3%)	378 (27.8%)	1581 (26.1%)	41 (100%)
Tree length	1781	533	1238	5225	371
CI	0.477	0.457	0.471	0.459	0.189
RI	0.675	0.601	0.623	0.645	0.441

were also provided by this data set. Of the other data sets, the *rpl16* and *rps16* introns performed equally well, with similar tree statistics and levels of resolution. The 3′ *trnK* intron provided the least-resolved cladogram, and *rbcL* provided the lowest proportion of parsimony-informative characters and CI and RI values. No strongly supported (> 75%) incongruent clades were found between individual partitions; therefore, all molecular data sets were combined, and only the combined tree is shown (Fig. 1). Analysis of the morphological data set resulted in a poorly resolved cladogram (not shown, available upon request from the first author).

For the Bayesian analysis, the model GTR + γ was selected for *rbcL* and the *rpl16*, *rps16*, and 3′ *trnK* introns, whereas the model TVM + γ was selected for *matK* by Modeltest. Parameter values of each molecular data set suggested by Modeltest are summarized in Table 2. Application of both parsimony and Bayesian methods to the molecular combined data set resulted in similar topologies, although posterior probability values (PP) for clades were higher in most cases than parsimony bootstrap values (Figs. 1, 2). All further discussion will be based on the strict consensus cladogram resulting from parsimony analysis of the molecular combined data set as well as the Bayesian tree obtained from the same data set.

Apocynaceae s.l. are strongly supported as monophyletic in both the MP and the Bayesian analyses (BS, PP = 100%), a result also supported by analysis of each individual molecular data set (except for the 3′ *trnK* intron), as well as the morphological data set. Within Apocynaceae, 13 strongly supported (BS, PP = 100%) clades were recovered, of which one, the APSA clade, included all exemplars of Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae. The remaining taxa form the paraphyletic Rauvolfioideae, a grade with its representatives dispersed among 12 remaining clades. Our selection of these clades was mainly based on their correspondence to the tribes in Rauvolfioideae recognized by Endress and Bruyns (2000): (1) the Aspidospermeae clade (ASP); (2) the Alstonieae clade (ALS); (3) the Vinceae clade (VIN); (4) the Willughbeieae clade (WIL); (5) the Tabernaemontaneae clade (TAB); (6) the Hunterieae clade (HUN); (7) *Diplorhynchus* (DI); (8) the Melodineae clade (MEL); (9) the *Amsonia* clade (AMS); (10) the Alyxieae clade (ALY); (11) the Plumerieae clade (PLU); and (12) the Carisseae clade (CAR).

CHARACTER MAPPING

Mapping morphological characters onto one of the most parsimonious trees showed that only four of the 11 major clades of Rauvolfioideae (Alyxieae, Aspidospermeae, Vinceae, and Tabernaemontaneae clades) are supported by unique, unambiguous changes. For most characters, reconstruction of character state changes resulted in ambiguous assumptions of ancestral state and numerous shifts in character state (Figs. 3, 4), even when different most parsimonious trees were selected for optimization. An example is given by character number 24, in which congenitally syncarpous ovaries were observed in all representatives of the Willughbeieae, Melodineae, and Carisseae clades, but also in one species of the Alyxieae clade (*Chilocarpus costatus* Miq.) and two from the Tabernaemontaneae clade (*Ambelania acida* Aubl. and *Molongum laxum* (Benth.) Pichon).

DISCUSSION

RELATIONSHIPS WITHIN RAUVOLFIOIDEAE

The structure of the combined molecular trees in Figures 1 and 2 is in general agreement with previously published phylogenies of Apocynaceae based on chloroplast DNA (e.g., Civeyrel et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002). Neither of the two subfamilies of Apocynaceae s. str. (Rauvolfioideae and Apocynoideae) is monophyletic. Rauvolfioideae, as traditionally defined, comprises the taxa from *Aspidosperma* Mart. & Zucc. through *Carissa* L. (Figs. 1, 2), forming a grade that is paraphyletic to the rest of the family. Periplocoideae, Secamonoideae, and Asclepiadoideae are nested within Apocynoideae, thus rendering it paraphyletic as well. The results of this study are compared here with those of Potgieter and Albert (2001) and Sennblad and Bremer (2002), and to the classification of Endress and Bruyns (2000).

Within Rauvolfioideae, most of the terminal clades retrieved in our analyses correspond to clades based on the *trnL* intron and the *trnL-F* spacer (Potgieter & Albert, 2001) and *rbcL* (Sennblad & Bremer, 2002) and, in most cases, equate to tribes in the current classification (sensu Endress & Bruyns, 2000). The support for these clades is, in general, higher here than in these previous studies. Resolution for the relationships among these clades at the tips of the branches is considerably improved as well, compared to the two earlier studies, especially in the region of the tree comprising the Aspidospermeae through the Tabernaemontaneae/Willughbeieae clades. The region of the tree (cf. Figs. 1, 2) including the Hunterieae, Melodineae, *Amsonia* Walter, and Alyxieae clades, as well as *Diplorhynchus* Welw. ex Ficalho & Hiern continues to remain unresolved.

The Aspidospermeae clade, comprising *Aspidosperma* (represented by *A. australe* Müll. Arg., *A.*

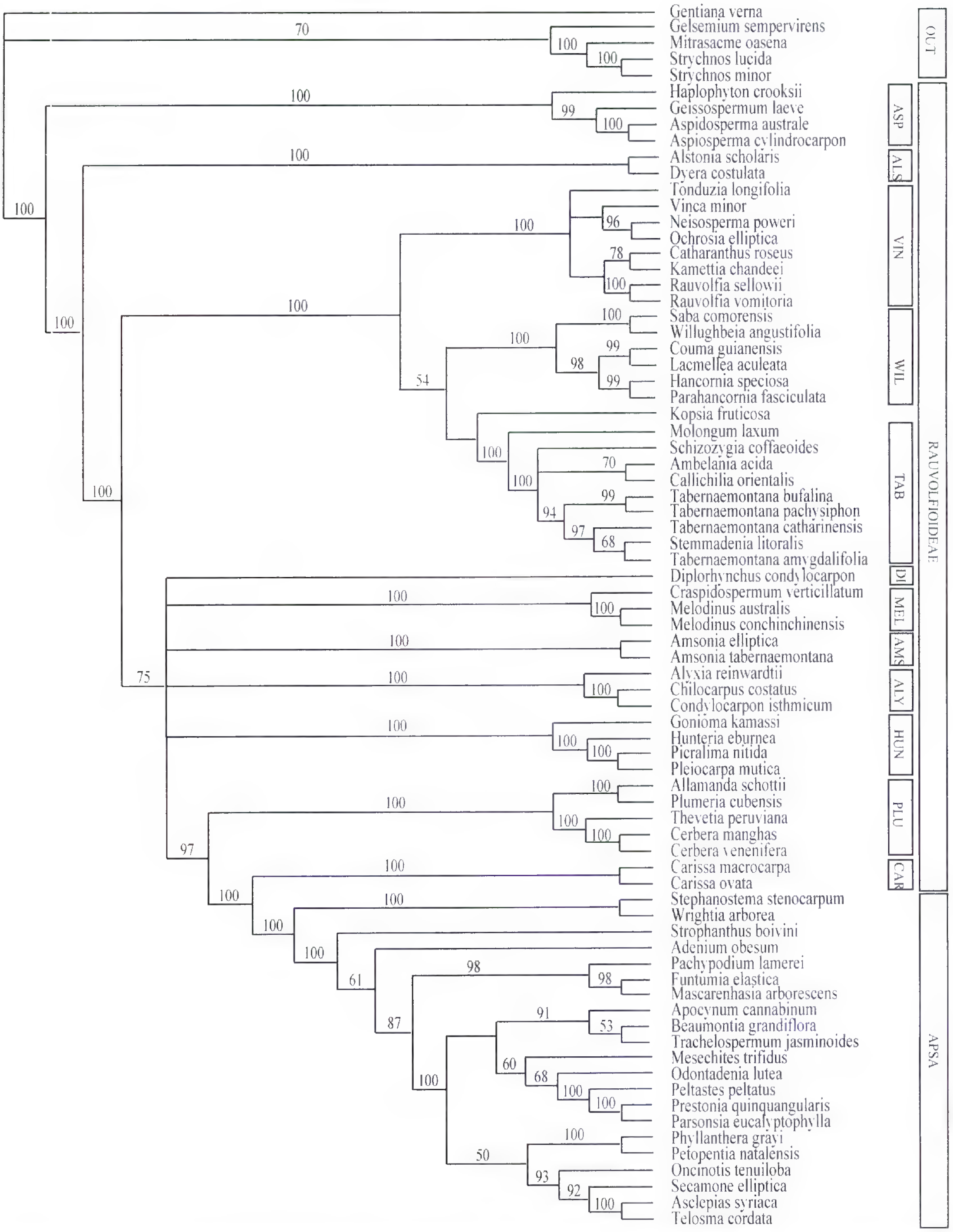

Figure 1. Strict consensus of the 28 most parsimonious trees generated by the molecular combined data set. Bootstrap values greater than 50% are indicated above the branches. Full taxon names are given in Appendix 1. The clades identified are abbreviated as follows: OUT = Outgroup; ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; WIL = Willughbeieae clade; TAB = Tabernaemontaneae clade; DI = *Dyplorhynchus*; MEL = Melodineae clade; AMS = *Amsonia* clade; ALY = Alyxieae clade; HUN = Hunterieae clade; PLU = Plumerieae clade; CAR = Carisseae clade; APSA = Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae.

Table 2. Summary of the model parameters suggested by Modeltest 3.06 for the molecular data sets.

Molecular data set	Substitution model	-ln L	Gamma shape parameter	Base frequency				Rate matrix					
				A	C	G	T	A-C	A-G	A-T	C-G	C-T	G-T
matK	TVM+I+G	14345.0674	1.0878	0.2897	0.1699	0.3699	0.1705	1.1761	1.7446	0.2101	0.8363	1.7446	1.0000
rbcL	GTR+I+G	7323.1772	0.7065	0.2135	0.2005	0.2915	0.2646	1.7303	2.2893	0.4607	1.1604	3.0710	1.0000
rpl16 intron	GTR+I+G	8850.9785	1.0623	0.1269	0.1330	0.2828	0.1574	1.0078	1.0078	0.3027	0.6600	1.3899	1.0000
rps16 intron	GTR+I+G	7388.5928	1.1501	0.3498	0.1521	0.3117	0.1831	1.0082	1.2571	0.3209	0.6133	1.7876	1.0000
3′trnK intron	GTR+G	2979.7278	3.0211	0.3759	0.1100	0.3218	0.1923	1.1913	1.1683	0.5878	1.1038	1.7375	1.0000

cylindrocarpon Müll. Arg.), *Geissospermum* Allemão (*G. laeve* Baill.), and *Haplophyton* A. DC. (*H. crooksii* L. D. Benson) sensu Alstonieae (Endress & Bruyns, 2000) and Aspidospermeae (Sennblad & Bremer, 2002), is strongly supported as sister to the rest of the family. The basal position of this clade of New World taxa, as well as the separation of the Old World genus *Alstonia* R. Br. (Alstonieae) as a distinct clade, concurs with the findings of Potgieter and Albert (2001). The three genera of the Aspidospermeae clade are characterized by having conspicuous slits in the corolla tube just above the level of stamen insertion (Appendix 3, char. 12: 1) that are usually visible under the dissecting scope or with the naked eye. These slits are formed when the flower reaches anthesis before the postgenital fusion of the upper part of the corolla tube is completed. Remnants of epidermis along the suture line or tiny gaps a few microns in length in this region, visible in serial sections and at high magnification, have been reported in various genera of both Rauvolfioideae and Apocynoideae (Endress et al., 1996; Sennblad et al., 1998). It is extremely rare, however, that the gaps are so long that they can be seen as slits under a normal dissecting scope. Visible slits in the corolla tube were considered to be a key character to distinguish *Aspidosperma* from related genera by Woodson (1951) and Markgraf (1974). However, visible slits in the corolla tube were also reported within the other two genera represented in the Aspidospermeae clade, *Geissospermum* and *Haplophyton*, by Fallen (1986).

The large and widespread Paleotropical genus *Alstonia*, also recognized as a clade in the analyses of Endress et al. (1996) and Potgieter and Albert (2001), here with *A. scholaris* R. Br., forms a strong sister relationship with *Dyera* Hook. f. (*D. costulata* Hook. f. included as exemplar), another genus of the Old World tropics containing only two species. Although currently included in the Melodineae, it has previously been suggested that *Dyera* is the closest relative of *Alstonia*, based on morphological similarities including the large tree habit, the presence of several leaves in a whorl, and an unusual type of outgrowth at the base of the petiole in both *Dyera* species and *A. scholaris* (Monachino, 1949; Sidiyasa, 1998; D. J. Middleton, pers. comm.).

The Vinceae clade (Figs. 1, 2) corresponds to previous Vinceae clades (Potgieter & Albert, 2001; Sennblad & Bremer, 2002). *Catharanthus* G. Don, *Rauvolfia* L., *Vinca* L., *Neisosperma* Raf., and *Ochrosia* Juss. are all members of Vinceae in Endress and Bruyns (2000). The current results also support inclusion of *Tonduzia* Pittier (*T. longifolia* (A. DC.) Woodson), previously classified in Alstonieae, and

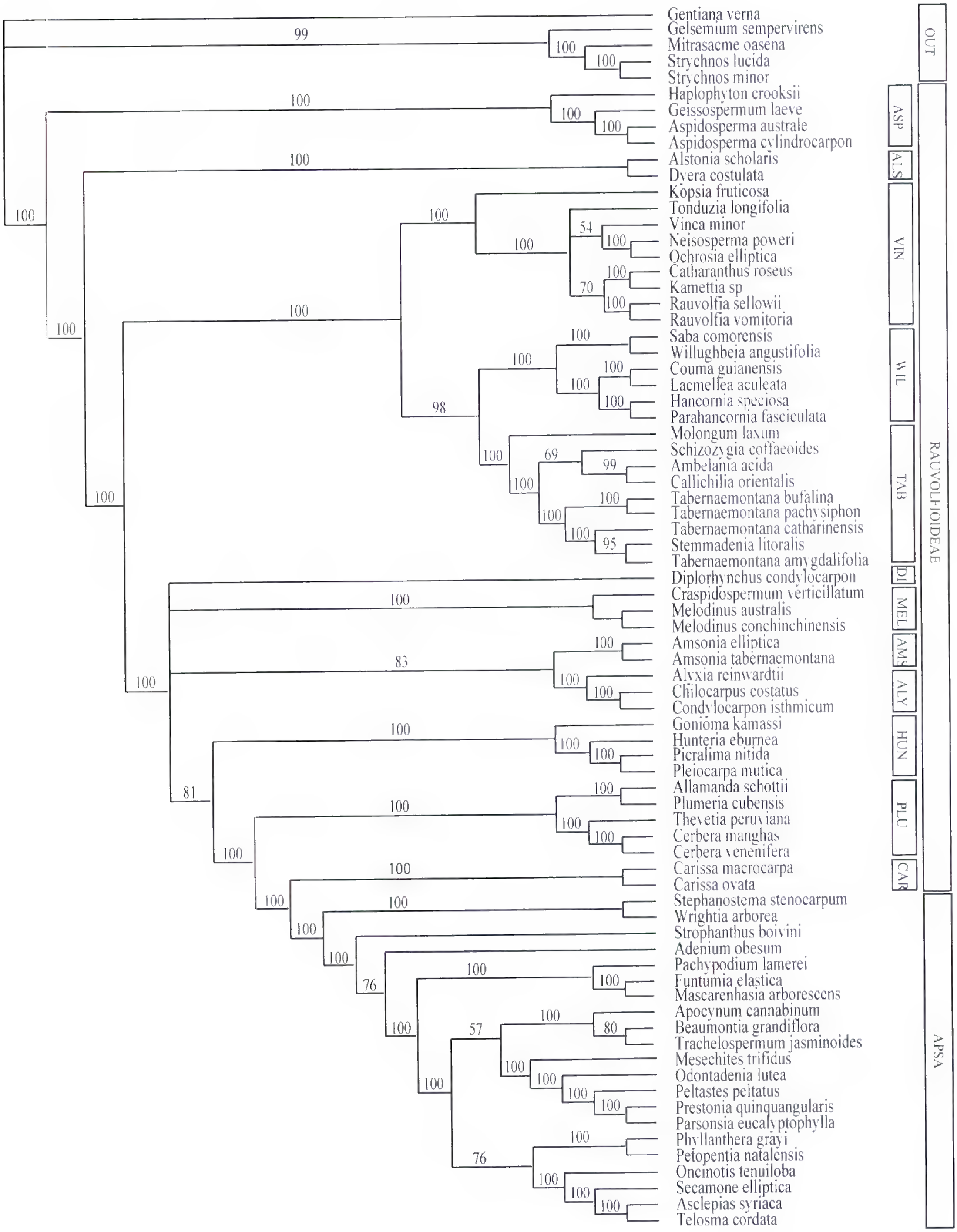

Figure 2. Majority rule consensus tree based on the Bayesian analysis of the combined molecular data set. Posterior probabilities are indicated above the branches. Full taxon names are given in Appendix 1. The clades identified are abbreviated as follows: OUT = Outgroup; ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; WIL = Willughbeieae clade; TAB = Tabernaemontaneae clade; DI = *Dyplorhynchus*; MEL = Melodineae clade; AMS = *Amsonia* clade; ALY = Alyxieae clade; HUN = Hunterieae clade; PLU = Plumerieae clade; CAR = Carisseae clade; APSA = Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae.

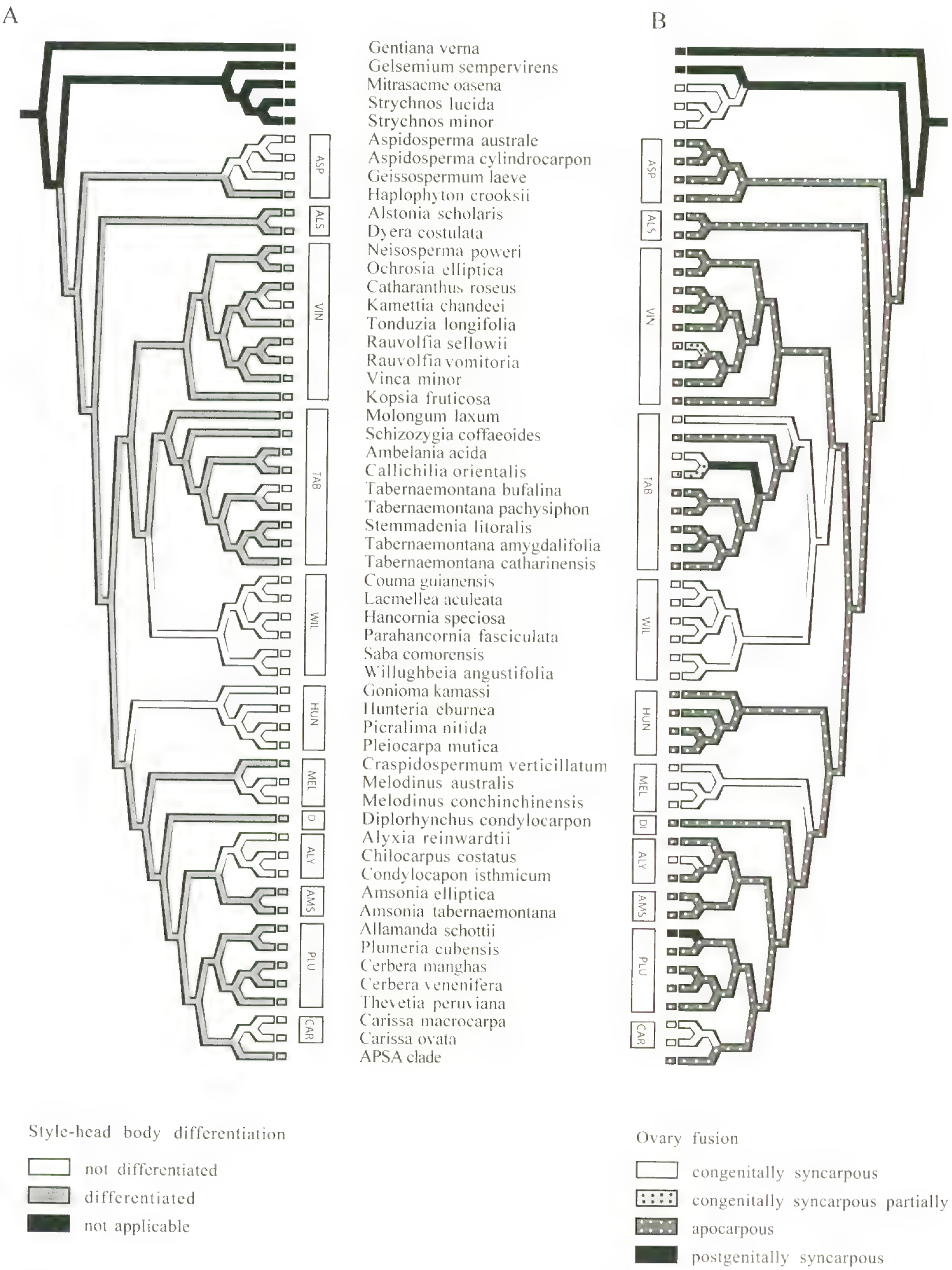

Figure 3. Evolution of two selected flower characters in Rauvolfioideae optimized onto one of the most parsimonious trees from the combined molecular data set. Only ACCTRAN optimization is shown. —A. Style-head body differentiation (char. 20). —B. Ovary fusion (char. 24). Clade names within Rauvolfioideae are abbreviated as follows: ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; TAB = Tabernaemontaneae clade; WIL = Willughbeieae clade; HUN = Hunterieae clade; MEL = Melodineae clade; DI = *Diplorynchus*; ALY = Alyxieae clade; AMS = *Amsonia* clade; PLU = Plumerieae clade; CAR = Carisseae clade.

Figure 4. Evolution of two selected fruit and seed characters optimized onto one of the most parsimonious trees from the combined molecular data set. Only ACCTRAN optimization is shown. —A. Fruit dehiscence (char. 26). —B. Seed margin (char. 29). Clade names within Rauvolfioideae are abbreviated as follows: ASP = Aspidospermeae clade: ALS = Alstonieae clade; VIN = Vinceae clade; TAB = Tabernaemontaneae clade; WIL = Willughbeieae clade; HUN = Hunterieae clade; MEL = Melodineae clade; DI = *Dyplorynchus*; ALY = Alyxieae clade; AMS = *Amsonia* clade; PLU = Plumerieae clade; CAR = Carisseae clade.

Kamettia Kostel., previously placed in Melodineae, in the Vinceae. *Tonduzia* was put into synonymy under *Alstonia* by Pichon (1947) and maintained there by subsequent authors (Gentry, 1983; Morales, 1995; Williams, 1996; Sidiyasa, 1998). In the Endress and Bruyns (2000) classification. *Tonduzia* is recognized as a distinct genus in Alstonieae. In the study by Potgieter and Albert (2001), *Tonduzia* as well as *Laxoplumeria* Markgr. were strongly supported as members of the Vinceae. *Kamettia*, a rare and poorly collected genus known previously from a single species in the Western Ghats of India, is represented in this study by *K. chandeei* D. J. Middleton, a new species discovered by fellow apocynologist David Middleton during fieldwork in Thailand (Middleton et al., 2005). *Kamettia* is currently included in Melodineae by Endress and Bruyns (2000) but is strongly supported by the one exemplar here as a member of Vinceae. The ongoing dispute over the status of *Neisosperma* as a genus distinct from *Ochrosia* based on morphological criteria (Fosberg & Sachet, 1977; Hendrian, 2004) could not be adequately tested here due to insufficient taxon sampling. The strongly supported sister relationship of the two included exemplars, however, attests to their close affinity from a molecular perspective and does not contradict their being congeners, as they have been treated by several specialists of Paleotropical Apocynaceae in more recent years (Leeuwenberg, 1994a; Hendrian, 2004; Middleton, 2004b). Therefore, we have decided to follow their lead and treat *Neiosperma* as a synonym of *Ochrosia*.

The position of *Kopsia* Blume, an Asian genus of some 24 species (Middleton, 2004a) currently included in Vinceae, could not be resolved satisfactorily in the parsimony analysis, in which *K. fruticosa* (Roxb.) A. DC. was sister to Tabernaemontaneae (but with $< 50\%$ bootstrap support). Bayesian inference placed *Kopsia* as sister to the rest of Vinceae with 100% support, a position very weakly supported in the study by Sennblad and Bremer (2002). Morphologically, *Kopsia* resembles other members of Vinceae in a number of characters. The style-head has a short membranous basal collar (Middleton, 2004a, b) and two well-developed nectary lobes alternating with the carpels, as also found in *Vinca*, *Catharanthus*, and *Laxoplumeria*; two much reduced nectaries in the same position are also present in *Neisosperma* (Forster & Williams, 1996). These characteristics are typical for Vinceae, but otherwise relatively uncommon in Rauvolfioideae. In addition, the corolla lobes are dextrorsely convolute, as found in *Ochrosia* (including *Neisosperma*), whereas in the great majority of Rauvolfioideae, the corolla lobes are sinistrorsely convolute. Chemotaxonomically, the presence of exclusively complex plumeran alkaloids with a rearranged secologanin backbone in *Kopsia* suggests a somewhat isolated position for the genus in Vinceae (Kisakürek et al., 1983; Homberger & Hesse, 1981). The co-occurrence of these characters in *Kopsia* is the basis for its inclusion in Vinceae in the classification of Endress and Bruyns (2000).

The Willughbeieae clade, comprising *Couma* Aubl. (*C. guianensis* Aubl.), *Hancornia* Gomez (*H. speciosa* Gomez), *Lacmellea* H. Karst. (*L. aculeata* (Ducke) Monach.), *Parahancornia* Ducke (*P. fasciculata* (Poir.) Benoist), *Saba* (Pichon) Pichon (*S. comorensis* (Bojer) Pichon), and *Willughbeia* Roxb. (*W. angustifolia* (Miq.) Markgr.), largely corresponds to clades found by Potgieter and Albert (2001) and Sennblad and Bremer (2002). All taxa included in the Willughbeieae clade in our study, as well as in the studies by Potgieter and Albert (2001) and Sennblad and Bremer (2002), are currently included in Willughbeieae sensu Endress and Bruyns (2000), strongly supporting the monophyly of the tribe. Whereas in Sennblad and Bremer (2002) the Willughbeieae clade contained only closely related African taxa and in Potgieter and Albert (2001) it remained unresolved, here it is divided into two strongly supported clades: a New World clade containing *Couma*, *Hancornia*, *Lacmellea*, and *Parahancornia*, all from South America, and an Old World clade containing *Saba* from Africa and *Willughbeia* from Asia. Willughbeieae have a pantropical distribution, and with 18 recognized genera (Endress & Bruyns, 2000), they are the second largest tribe in Rauvolfioideae. The tribe comprises mainly trees in the New World and mostly robust lianas in the Old World. In most Old World species as well as *Pacouria* Aubl. in the New World, the inflorescences are modified into grappling, curled tendrils (found nowhere else in the family), allowing them to climb high into the canopy (char. 6: 1). The tribe is quite homogeneous and characterized by the congenitally syncarpous, mostly unilocular, fruits with parietal placentation, opposite leaves, long, slender, pale-colored corollas, and undifferentiated style-heads. Unlike most Apocynaceae, the fruits of Willughbeieae are almost always edible (Monachino, 1943; Leeuwenberg & van Dilst, 1989; Vonk, 1989; Zarucchi, 1991; Persoon et al., 1992; Vonk et al., 1994).

The broader circumscription of Tabernaemontaneae as delimited in the classification by Endress and Bruyns (2000), including Ambelanieae of earlier classifications and corroborated by Potgieter and Albert (2001) and Sennblad and Bremer (2002), is also strongly supported here by the inclusion of *Molongum* Pichon (*M. laxum* (Benth.) Pichon) and *Ambelania* Aubl. (*A. acida* Aubl.) in the Tabernaemontaneae clade. Tabernaemontaneae is the largest

tribe in the Rauvolfioideae and is also the most phenotypically homogeneous. With a few rare exceptions, all genera are trees with opposite leaves and the fruit a pair of follicles. The two most distinctive character states typically found in Tabernaemontaneae are the highly modified anthers with large lignified guide rails (char. 16: 1; found nowhere else in Rauvolfioideae) and the arillate seeds (char. 28: 1). The only other genus in Apocynaceae known to have arillate seeds is *Chilocarpus* Blume of the Alyxieae. These arils are a fleshy, cushion-like outgrowth restricted to the funicle, whereas in Tabernaemontaneae, the pulpy aril surrounds the seed (Corner, 1976a, b). *Tabernaemontana* L., as circumscribed by Leeuwenberg (1994b), is a large pantropical genus of some 110 species, divided into seven sections. The species included in our study represent four of these sections, but they have also been treated earlier as separate genera: *T. bufalina* Lour. as *Ervatamia* (A. DC.) Stapf, *T. pachysiphon* Stapf as *Conopharyngia* G. Don, *T. catharinensis* A. DC. as *Peschiera* A. DC., and *T. amygdalifolia* Sieber ex A. DC. as *Tabernaemontana* s. str. (Stapf, 1902; Allorge, 1985). Our results suggest paraphyly of *Tabernaemontana*, as the four sampled species of the genus form a strongly supported clade with the sampled species of *Stemmadenia* Benth. (*S. litoralis* (Kunth) L. Allorge).

A sister relationship of the Willughbeieae and Tabernaemontaneae clades was supported in both the MP and Bayesian analyses, although this topology received a bootstrap support of only 54% in the parsimony tree.

Sister to the Tabernaemontaneae, Willughbeieae, and Vinceae clades is a polytomy composed of six clades: *Diplorhynchus*, the Melodineae clade, the *Amsonia* clade, the Alyxieae clade, the Hunterieae clade, and a large clade composed of the Plumerieae, Carisseae, and APSA clades (Fig. 2).

Diplorhynchus, a monotypic genus restricted to southern Africa, has traditionally been considered to be closely related to *Pycnobotrya* Benth., *Aspidosperma*, *Geissospermum*, and *Microplumeria* Baill. based on similarities of their fruit and seeds (Pichon, 1950; Plazier, 1980; van der Ploeg, 1983). Endress and Bruyns (2000) placed the last three genera in Alstonieae, whereas *Diplorhynchus* and *Pycnobotrya* are included in Melodineae. Although *Diplorhynchus condylocarpon* (Müll. Arg.) Pichon is part of an unresolved polytomy in our analyses, its position would preclude a close relationship with *Aspidosperma*. We hypothesize the closest relative of *Diplorhynchus* may be *Pycnobotrya*, another monotypic and African genus. Both genera have latex that is often yellow, terminal, many-flowered inflorescences, anthers with sterile appendages at the base and apex, apocarpous ovary with four seeds per carpel, and stout, widely divergent follicular fruits and compressed, winged seeds. Our attempts to obtain leaf samples of *Pycnobotrya* failed, and future molecular studies should make every effort to include this genus.

The Melodineae clade, representing Melodineae sensu Endress and Bruyns (2000), was also retrieved in previous analyses (Potgieter & Albert, 2001; Sennblad & Bremer, 2002). The strongly supported sister relationship of *Craspidospermum verticillatum* Bojer with two species of *Melodinus* is difficult to understand morphologically. Whereas the monotypic Madagascan endemic, *Craspidospermum* Bojer ex A. DC., is a tree with leaves in whorls of three to four, flowers without a petaloid corona, and dry capsular fruits, species of *Melodinus* J. R. Forst. & G. Forst., a genus of about 25 species with a wide distribution in Asia and Australasia (D. Middleton, pers. comm.), are climbers with opposite (rarely ternate) leaves, flowers with a conspicuous petaloid corona in the throat, and indehiscent berries with the seeds embedded in pulp. The porate pollen of *Craspidospermum*, shed as pantoporate tetrads (Lienau et al., 1986), is otherwise unknown in Rauvolfioideae and superficially resembles the pollen of some Periplocoideae (Nilsson et al., 1993). The Melodineae is the most poorly understood tribe in Rauvolfioideae, and although *Melodinus* was recently revised (Leeuwenberg, 2003), the treatment is unsatisfactory.

The position of the *Amsonia* clade here is at odds with its previous inclusion in Vinceae (Endress & Bruyns, 2000). *Amsonia* was also distinct from Vinceae in the analysis of Potgieter and Albert (2001). Its suggested relationship to *Thevetia* L. in that study may be incorrect, however, because both here and in other studies (Endress et al., 1996; Sennblad & Bremer, 1996, 2002; Civeyrel et al., 1998; Alvarado-Cárdenas & Ochoterena, 2007, this issue) *T. peruviana* is shown to be related to genera in the Plumerieae. Morphological support for the position of *Amsonia* as sister to the Alyxieae clade herein (Fig. 2) is lacking at present. The short collar at the base of the style-head and slender dehiscent follicles with naked seeds (e.g., Rosatti, 1989: 360) make *Amsonia* morphologically similar to some Vinceae (e.g., *Catharanthus* and *Vinca*). The genus needs comprehensive study.

All members of the Alyxieae clade here (*Alyxia reinwardti* Blume, *Chilocarpus costatus*, and *Condylocarpon isthmicum* (Vell.) A. DC.) agree with previous results (Potgieter & Albert, 2001; Sennblad & Bremer, 2002) and belong to the Alyxieae sensu Endress and Bruyns (2000), which is composed of *Alyxia* Banks ex R. Br., a large genus (100+ species) widespread in

Asia and Australasia (Middleton, 2000, 2002), and six small genera, four of them island endemics (Lorence & Wagner, 1997; Endress et al., 1997, 2007). Morphologically, this tribe is best characterized by its unusual pollen. The pollen apertures are large pores, usually with a thickened margin, and are irregularly spaced on the grain (char. 36: 1). This is not homologous to the porate pollen found in the other subfamilies and is restricted to the Alyxieae (van der Ham et al., 2001). A sister relationship between the *Amsonia* and Alyxieae clades was retrieved in the Bayesian tree, but this topology received only moderate support (PP = 83%).

The Hunterieae clade corresponds to similar clades found by both Potgieter and Albert (2001) and Sennblad and Bremer (2002) and the Hunterieae sensu Endress and Bruyns (2000); it is comprised of the three genera: *Hunteria* Roxb. (*H. eburnea* Pichon), *Picralima* Pierre (*P. nitida* Th. & H. Dur.), and *Pleiocarpa* Benth. (*P. mutica* Benth.), all African. *Gonioma* E. Mey., a genus of two species restricted to South Africa and Madagascar, characterized by dehiscent fruits with small winged seeds and currently included in Melodineae, is strongly supported here by *G. kamassi* E. Mey. in the Hunterieae clade, in which all other genera have fleshy, indehiscent fruits. The Hunterieae clade received moderate support (PP = 81%) as sister to the next higher clade (composed of Carisseae, Plumerieae, and the APSA clades) in the Bayesian tree, but this was not retrieved in the strict consensus tree of the MP analysis.

Our phylogenetic analyses support the monophyly of Plumerieae, as circumscribed by Endress and Bruyns (2000). As noted, the position of *Thevetia peruviana* as sister to *Amsonia* in the study by Potgieter and Albert (2001) is most likely due to human error, because *T. peruviana* comes out together with the other members of Plumerieae, as expected, here, as well as in previous studies (Endress et al., 1996; Civeyrel et al., 1998; Sennblad & Bremer, 2002). In a detailed morphological cladistic study including all eight recognized species of *Thevetia* (including *Cascabela* Raf.), all taxa formed a clade (Alvarado-Cárdenas, 2005; Alvarado-Cárdenas & Ochoterena, 2007).

Molecular studies have greatly altered our understanding of the phylogenetic relationships of the genera traditionally included in the Carisseae. Historically considered to contain the most plesiomorphic taxa in the family (e.g., Leeuwenberg, 1994a), Carisseae in the conventional sense (e.g., Pichon, 1948a; Leeuwenberg, 1994a) is polyphyletic here, with its constituent genera scattered among four clades (see Table 3). The position of Carisseae sensu Endress and Bruyns (2000) comprising only *Carissa* and *Acokanthera* G. Don as sister to the Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae concurs with results of Civeyrel et al. (1998) and Potgieter and Albert (2001). The highly nested position of *Carissa* (here *C. macrocarpa* A. DC., *C. ovata* R. Br.) and *Acokanthera* (Potgieter & Albert, 2001) indicates that characters that had been interpreted as plesiomorphic (e.g., the undifferentiated style-head and fleshy, indehiscent fruits) are derived in these two genera. Fleshy fruits are commonly associated with dispersal by animals, but observations on dispersal mode are lacking, not only for taxa of Carisseae, but for most other fleshy-fruited genera in the family. Comparative studies of the pollination biology and fruit dispersal are necessary to understand their functional and adaptive significance.

PHYLOGENY COMPARED TO PREVIOUS CLASSIFICATIONS

In Table 3, the relationships of the genera included in the present study are compared to their tribal placement in the two most recent classifications—Leeuwenberg (1994a) for the Apocynaceae s. str. and Endress and Bruyns (2000) for Apocynaceae s.l. Leeuwenberg's classification of Rauvolfioideae was based mainly on fruit and seed characters, and it recognized nine tribes: Carisseae, Chilocarpeae, Ambelanieae, Macoubeae, Tabernaemontaneae, Plumerieae, Alyxieae, Cerbereae, and Allamandeae, three of which (Chilocarpeae, Macoubeae, and Allamandeae) are monogeneric. Of the six remaining tribes, only one, Cerbereae (including *Cerbera* and *Thevetia*, nested in the Plumerieae clade), was found to be monophyletic, and five (Carisseae, Ambelanieae, Tabernaemontaneae, Plumerieae, and Alyxieae) were found to be nonmonophyletic. Alyxieae, Carisseae, and Plumerieae sensu Leeuwenberg (1994a) represented the most extreme cases of polyphyly here, with their constituent genera scattered across several clades in Rauvolfioideae, results that concur with those of Endress et al. (1996), Civeyrel et al. (1998), Sennblad et al. (1998), Potgieter and Albert (2001), and Sennblad and Bremer (2002).

The classification of Apocynaceae s.l. proposed by Endress and Bruyns (2000) also recognized nine tribes in Rauvolfioideae, but their circumscription differed significantly from that of Leeuwenberg. Tribal delimitation was based on floral, fruit and seed, and pollen characters, supplemented, when possible, by molecular phylogenetic results. Our results, corroborating the phylogenies obtained by Potgieter and Albert (2001) and Sennblad and Bremer (2002), show that the tribal classification of Rauvolfioideae by Endress and Bruyns (2000) contains more mono-

phyletic groups than that of Leeuwenberg. Six of the nine tribes were found to be monophyletic (Willughbeieae, Tabernaemontaneae, Hunterieae, Alyxieae, Plumerieae, and Carisseae), and three were found to be polyphyletic (Alstonieae, Melodineae, and Vinceae). Melodineae represented the most extreme case of polyphyly, with its genera scattered across five different clades.

MORPHOLOGICAL HOMOPLASY

The mapping of morphological character state changes onto one of the most parsimonious trees obtained from the combined molecular matrix indicates extensive morphological homoplasy among the characters that were traditionally used to circumscribe Rauvolfioideae. Fruit and seed characters, in particular, seem to be especially affected by homoplasy. The following characters are examined, because they were considered to be key features in traditional classifications: style-head body differentiation, ovary fusion, fruit dehiscence, and seed margin.

- Style-head body differentiation (char. 20). A style-head body with a simple ovoid or globular shape and a non-differentiated, uniformly secretory epithelium that functions both as stigma and for production of pollen transport adhesive (Appendix 3) is characteristic for certain genera of Rauvolfioideae. Optimization of character state changes on all selected most parsimonious trees produced similar unambiguous reconstructions. Non-differentiated style-heads have arisen independently eight times in taxa scattered throughout the Rauvolfioideae, from the basalmost Aspidospermeae clade (except *Haplophyton*) to the most derived Carisseae clade (Fig. 3A). Concerted switches to non-differentiated style-heads, syncarpy, and indehiscent fruits co-occurred along the same branches leading to Willughbeieae, *Melodinus*, and Carisseae.
- Ovary fusion (char. 24). In traditional classifications, syncarpy was thought to be the primitive condition in the family, and most taxa with syncarpous ovaries were grouped into Carisseae, which was placed at the base of the family (Bentham & Hooker, 1876; Schumann, 1895; Pichon, 1949; Li, 1982; Lý, 1986; Leeuwenberg, 1994a). The plesiomorphic condition in the family is apocarpy, as previously suggested by Endress et al. (1996) and Potgieter and Albert (2001). Shifts from apocarpous to syncarpous ovaries (Fig. 3B) have occurred in parallel five to six times in Rauvolfioideae. Evolution of this character within the Tabernaemontaneae–Willughbeieae clade is equivocal, and reconstruction of character state changes resulted in two equally parsimonious hypotheses. In the first, syncarpy is the apomorphic state, with frequent reversals to apocarpous ovaries. Alternatively, syncarpous ovaries could have evolved independently three times, once in the Willughbeieae clade and twice in the Tabernaemontaneae clade (demonstrated once by *Molongum laxum* and again in *Ambelania acida*). A broader sampling of Tabernaemontaneae (sensu Endress & Bruyns, 2000), especially from genera with syncarpous ovaries, such as *Rhigospira* and *Spongiosperma*, is necessary for a better understanding of this character in the Tabernaemontaneae–Willughbeieae clade.
- Fruit dehiscence (char. 26). Fruit dehiscence also shows considerable homoplasy, underscoring the evolutionary lability in this character (Fig. 4A). From an ancestral state of dehiscent fruits, shifts to indehiscent fruits have occurred six to 11 times within Rauvolfioideae. This remarkable discrepancy in the number of hypothesized changes may be due to the weakly resolved phylogenetic relationships between the Alyxieae, *Amsonia*, Hunterieae, and Melodineae clades, plus *Diplorhynchus*. In one of the most parsimonious trees, reconstruction of character state changes resulted in one single origin of indehiscent fruits with several subsequent reversals to dehiscent fruits in this group, whereas four independent origins of indehiscent fruits can be equally reconstructed when a different most parsimonious tree is used as a template. The switches to syncarpy and indehiscence often occurred along the same branches in our optimizations. Almost all of the genera included in the Carisseae in traditional classifications have congenitally syncarpous berries. The exceptions are *Hunteria*, *Picralima*, and *Pleiocarpa*. These three genera formed the subtribe Pleiocarpinae of the Carisseae in traditional classifications (Pichon, 1948a; Leeuwenberg, 1994a; Omino, 1996), which corresponds to the core Hunterieae clade here. The berries in these genera are superficially similar to those in Willughbeieae. However, because these taxa are apocarpous, each carpel in the gynoecium matures into a separate berry. In the case of *Pleiocarpa*, one of the few genera of Apocynaceae in which some species have a gynoecium with more than two carpels, the fruit can be composed of up to five berries per flower. The congenitally syncarpous genus *Carissa*, the epitome of the primitive Apocynaceae (Leeuwenberg, 1994a) in traditional classifications, is now found to be well embedded in the phylogeny of Apocynaceae, as sister to higher Apocynaceae (Figs. 1, 2).

Table 3. Comparison of the classifications of Leeuwenberg (1994a) and Endress and Bruyns (2000) for taxa considered within Apocynaceae with the current phylogeny. NA = not applicable.

Genera	Leeuwenberg (1994a)	Endress & Bruyns (2000)	This paper
RAUVOLFIOIDEAE			
Allamanda L.	Allamandeae	Plumerieae	Plumerieae clade
Alstonia R. Br.	Plumerieae	Alstonieae	Alstonieae clade
Alyxia Banks ex R. Br.	Alyxieae	Alyxieae	Alyxieae clade
Ambelania Aubl.	Ambelanieae	Tabernaemontaneae	Tabernaemontaneae clade
Amsonia Walter	Plumerieae	Vinceae	*Amsonia* clade
Aspidosperma Mart. & Zucc.	Plumerieae	Alstonieae	Aspidospermeae clade
Callichilia Stapf	Tabernaemontaneae	Tabernaemontaneae	Tabernaemontaneae clade
Carissa L.	Carisseae	Carisseae	Carisseae clade
Catharanthus G. Don	Plumerieae	Vinceae	Vinceae clade
Cerbera L.	Cerbereae	Plumerieae	Plumerieae clade
Chilocarpus Blume	Chilocarpeae	Alyxieae	Alyxieae clade
Condylocarpon Desf.	Alyxieae	Alyxieae	Alyxieae clade
Couma Aubl.	Carisseae	Willughbeieae	Willughbeieae clade
Craspidospermum Bojer ex A. DC.	Plumerieae	Melodineae	Melodineae clade
Diplorhynchus Welw. ex Ficalho & Hiern	Plumerieae	Melodineae	*Diplorhynchus* clade
Dyera Hook. f.	Plumerieae	Melodineae	Alstonieae clade
Geissospermum Allemão	Plumerieae	Alstonieae	Aspidospermeae clade
Gonioma E. Mey.	Plumerieae	Melodineae	Hunterieae clade
Hancornia Gomes	Carisseae	Willughbeieae	Willughbeieae clade
Haplophyton A. DC.	Plumerieae	Alstonieae	Aspidospermeae clade
Hunteria Roxb.	Carisseae	Hunterieae	Hunterieae clade
Kamettia Kostel.	Plumerieae	Melodineae	Vinceae clade
Kopsia Blume	Alyxieae	Vinceae	Vinceae clade
Lacmellea H. Karst.	Carisseae	Willughbeieae	Willughbeieae clade
Melodinus J. R. Forst. & G. Forst.	Carisseae	Melodineae	Melodineae clade
Molongum Pichon	Ambelanieae	Tabernaemontaneae	Tabernaemontaneae clade
Neisosperma Raf.	NA	Vinceae	Vinceae clade
Ochrosia Juss.	Alyxieae	Vinceae	Vinceae clade
Parahancornia Ducke	Carisseae	Willughbeieae	Willughbeieae clade
Picralima Pierre	Carisseae	Hunterieae	Hunterieae clade
Pleiocarpa Benth.	Carisseae	Hunterieae	Hunterieae clade
Plumeria L.	Plumerieae	Plumerieae	Plumerieae clade
Rauvolfia L.	Alyxieae	Vinceae	Vinceae clade
Saba (Pichon) Pichon	Carisseae	Willughbeieae	Willughbeieae clade
Schizozygia Baill.	Tabernaemontaneae	Tabernaemontaneae	Tabernaemontaneae clade
Stemmadenia Benth.	Tabernaemontaneae	Tabernaemontaneae	Tabernaemontaneae clade
Tabernaemontana L.	Tabernaemontaneae	Tabernaemontaneae	Tabernaemontaneae clade
Thevetia L.	Cerbereae	Plumerieae	Plumerieae clade
Tonduzia Pittier	Plumerieae	Alstonieae	Vinceae clade
Vinca L.	Plumerieae	Vinceae	Vinceae clade
Willughbeia Roxb.	Carisseae	Willughbeieae	Willughbeieae clade
APOCYNOIDEAE			
Adenium Roem. & Schult.	Wrightieae	Wrightieae	APSA clade
Apocynum L.	Apocyneae	Apocyneae	APSA clade
Beaumontia Wall.	Wrightieae	Apocyneae	APSA clade
Funtumia Stapf	Wrightieae	Malouetieae	APSA clade
Mascarenhasia A. DC.	Wrightieae	Malouetieae	APSA clade
Mesechites Müll. Arg.	Echiteae	Mesechiteae	APSA clade
Odontadenia Benth.	Echiteae	Apocyneae	APSA clade
Oncinotis Benth.	Apocyneae	Apocyneae	APSA clade
Pachypodium Lindl.	Echiteae	Malouetieae	APSA clade
Parsonsia R. Br.	Echiteae	Echiteae	APSA clade
Peltastes Woodson	Echiteae	Echiteae	APSA clade

Table 3. Continued.

Genera	Leeuwenberg (1994a)	Endress & Bruyns (2000)	This paper
Prestonia R. Br.	Echiteae	Echiteae	APSA clade
Stephanostema K. Schum.	Wrightieae	Wrightieae	APSA clade
Strophanthus DC.	Wrightieae	Wrightieae	APSA clade
Trachelospermum Lem.	Echiteae	Apocyneae	APSA clade
Wrightia R. Br.	Wrightieae	Wrightieae	APSA clade
PERIPLOCOIDEAE			
Petopentia Bullock	NA	Periplocoideae	APSA clade
Phyllanthera Blume	NA	Periplocoideae	APSA clade
SECAMONOIDEAE			
Secamone R. Br.	NA	Secamonoideae	APSA clade
ASCLEPIADOIDEAE			
Asclepias L.	NA	Asclepiadoideae	APSA clade
Telosma Coville	NA	Asclepiadoideae	APSA clade

- Seed margin (char. 29). The seeds of many genera in Rauvolfioideae have a wing or wings at the margin, and this is a key character for Plumerieae in Leeuwenberg's (1994a) classification. These wings are variable, and assessing their development requires detailed ontogenetic studies. Some taxa have a single, concentric, usually papery, wing (e.g., *Dyera*, *Pycnobotrya*, *Stephanostegia* Baill., many *Aspidosperma*). Other genera are characterized by seeds with a wing at only one end (*Plumeria* Tourn. ex L., *Diplorhynchus*, some *Aspidosperma*) or a wing at each end (e.g., *Gonioma*, *Kamettia*, *Plectaneia* Thou., *Strempeliopsis* Benth.). In *Craspidospermum*, the margin of the seed is completely surrounded by peculiar flattened appendages (see Leeuwenberg, 1997: fig. 1), here interpreted as a special type of wing (Appendix 3).

Mapping character state changes shows that wings have evolved several times within Rauvolfioideae, with six to seven independent origins of winged seeds observed in our reconstructions (Fig. 4B). This variation is due to the unstable position of *Diplorhynchus*, which had three different placements in our selected subset of most parsimonious trees. Switches to winged seeds and to dehiscent fruits occurred, not surprisingly, along the same branches. In *Cameraria* L. and *Cerberiopsis* Vieill. ex Pancher & Sebert (both Plumerieae), the fruit itself, an indehiscent samaroid, develops a wing.

Only three genera of Rauvolfioideae—*Alstonia*, *Tonduzia*, and *Laxoplumeria*—have flat seeds with a ciliate or fimbriate margin, which are not considered to be homologous to winged or comose seeds and are coded separately (Appendix 3). Because this type of seed is so rare in the family, taxonomists considered these three genera to be closely related. Pichon (1947) assigned *Tonduzia* to its own section within *Alstonia*, and with *Laxoplumeria* (as *Bisquamaria* Pichon) as a sister genus, and this was followed by subsequent specialists (Morales, 1995; Williams, 1996; Sidiyasa, 1998). Not until 2001, in the paper by Potgieter and Albert, were *Tonduzia* and *Laxoplumeria* shown to be unrelated to *Alstonia*.

The existence of different types of syncarpous, fleshy fruits and of winged seeds within Rauvolfioideae and their repeated evolution, as inferred from the molecular phylogeny (Figs. 3, 4), most likely correlate with fruit and seed dispersal syndromes. This may explain why traditional tribal circumscriptions based on interpretation of superficial fruit and seed characters in Rauvolfioideae resulted in nonmonophyletic groups, because they likely subsumed different morphological, evolutionary, and developmental pathways. Similar recurrent fruit and seed types in higher-level classification have been reported in a number of other tropical woody plant families, e.g., Myrtaceae (Johnson & Briggs, 1984); Rubiaceae (Bremer & Eriksson, 1992); Gesneriaceae (Smith & Carroll, 1997); Malvales (Alverson et al., 1999); and Melastomataceae (Clausing et al., 2000).

TAXONOMIC CONSEQUENCES

The following taxonomic changes (Appendix 4) are proposed to deal with paraphyly at the tribal level within Rauvolfioideae: (1) recognition of the Aspidospermeae Miers as a tribe separate from Alstonieae; (2) recognition of *Dyera* within the Alstonieae, of *Gonioma* within the Hunterieae, and of *Laxoplumeria*, *Tonduzia*, and *Kamettia* within the Vinceae; (3) and finally, the recognition of *Amsonia* and *Diplorhynchus*, originally included in Vinceae and Melodineae, respectively, as incertae sedis.

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Appendix 1. Species sequenced for the present study, voucher information, and GenBank accession numbers. For previously published sequences, EMBL Nucleotide Sequence Database numbers are not given here; instead the publication is cited by a letter as follows: A = Olmstead et al. (1993); B = Sennblad and Bremer (1996); C = Endress et al. (1996); D = Sennblad et al. (1998); E = Sennblad and Bremer (2002); F = Simões et al. (2004); G = Simões et al. (2006) (see Materials and Methods).

Species	Voucher	GenBank accession numbers				
		rpl16 intron	*rps16* intron	3′ *trnK* intron	*matK*	*rbcL*
INGROUP – APOCYNACEAE						
Adenium obesum (Forssk.) Roem. & Schult.	cult., U.S.A., Cornell Univ., *T. Livshultz 03-21* (BH)	DQ660746	DQ660556	DQ660678	DQ660494	D
Allamanda schottii Pohl	cult., U.S.A., Cornell Univ., *T. Livshultz 03-26* (BH)	DQ660747	DQ660557	DQ660679	DQ660495	DQ660626
Alstonia scholaris (L.) R. Br.	Sabah, *S. J. Davies et al. SJD99150* (A)	DQ660748	DQ660558	DQ660680	C	B
Alyxia reinwardtii Blume	Thailand, *D. J. Middleton 172* (A)	DQ660749	DQ660559	DQ660681	DQ660496	DQ660627
Ambelania acida Aubl.	French Guiana, *M. F. Prévost 3799* (CAY, P, Z)	DQ660750	DQ660560	DQ660682	DQ660497	DQ660628
Amsonia elliptica (Thumb.) Roem. & Schult.	cult., Netherlands, Bot. Gard. Wageningen, *acc. nr. 1991BG19812*	DQ660751	DQ660561	DQ660683	DQ660498	DQ660629
Amsonia tabernaemontana Walter	cult., Switzerland, Bot. Gard. Zurich, *A. O. Simões 1330* (Z)	DQ660752	DQ660562	DQ660684	DQ660499	DQ660630
Apocynum cannabinum L.	U.S.A., *T. Livshultz 03-28* (BH)	DQ660753	DQ660563	DQ660685	DQ660500	B
Asclepias syriaca L.	U.S.A., *T. Livshultz 03-33* (A)	DQ660754	DQ660564	DQ660686	DQ660501	DQ660631
Aspidosperma australe Müll. Arg.	Brazil, *L. S. Kinoshita 02/110* (UEC)	DQ660755	DQ660565	DQ660687	DQ660502	DQ660632
Aspidosperma cylindrocarpon Müll. Arg.	cult., Brazil, University of Campinas, *A. O. Simões 1328* (UEC)	DQ660756	DQ660566	DQ660688	DQ660503	DQ660633
Beaumontia grandiflora Wall.	cult., Germany, Bot. Gard. Munich, *G. Gerlach/J. Babczinsky 5/06* (M).	F	F	G	G	D
Callichilia orientalis S. Moore	cult., South Africa, *A. Hutchings s.n.* (ZULU 4000)	DQ660757	DQ660567	DQ660689	DQ660504	DQ660634
Carissa macrocarpa (Eckl.) A. DC.	cult., Switzerland, Bot. Gard. Zürich, *A. O. Simões 1331* (Z)	DQ660758	DQ660568	DQ660690	DQ660505	DQ660635
Carissa ovata R. Br.	Australia, *P. I. Forster 28689* (BRI)	DQ660759	DQ660569	DQ660691	DQ660506	DQ660636
Catharanthus roseus (L.) G. Don	cult., Brazil, University of Campinas, *A. O. Simões 1329* (UEC)	DQ660760	DQ660570	DQ660692	DQ660507	B
Cerbera manghas L.	Thailand, *D. J. Middleton et al. 2047* (A)	DQ660761	DQ660571	DQ660693	DQ660508	DQ660637
Cerbera venenifera (Poir.) Steud.	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S526* (BR)	DQ660762	DQ660572	DQ660694	DQ660509	E
Chilocarpus costatus Miq.	Thailand, *D. J. Middleton et al. 370* (A)	DQ660763	DQ660573	DQ660695	DQ660510	DQ660638
Condylocarpon isthmicum (Vell.) A. DC.	Brazil, *L. S. Kinoshita et al. 98-267* (UEC)	DQ660764	DQ660574	DQ660696	DQ660511	DQ660639
Couma guianensis Aubl.	French Guiana, *M. F. Prévost 3798* (CAY)	DQ660765	DQ660575	DQ660697	DQ660512	DQ660640
Craspidospermum verticillatum Bojer ex A. DC.	Madagascar, *Schönenberger et al. D36* (UPS)	DQ660766	DQ660576	DQ660698	DQ660513	DQ660641
Diplorhynchus condylocarpon (Müll. Arg.) Pichon	South Africa, 21 Apr. 2000, *E. Schmidt s.n.* (PRU)	DQ660767	DQ660577	DQ660699	DQ660514	E
Dyera costulata Hook. f.	cult., Singapore, Bot. Gard. Singapore, 23 May 2000, *Ian Turner s.n.* (SING)	DQ660768	DQ660578	DQ660700	DQ660515	DQ660642
Funtumia elastica (Preuss) Stapf	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S3927* (BR)	DQ660769	DQ660579	DQ660701	DQ660516	D
Geissospermum laeve (Vell.) Miers	French Guiana, *M. F. Prévost 3802* (CAY, P, Z)	DQ660770	DQ660580	DQ660702	DQ660517	DQ660643
Gonioma kamassi E. Mey.	South Africa, *T. Abbott 7713* (PRU, Z)	DQ660773	DQ660583	DQ660704	DQ660518	DQ660645
Hancornia speciosa Gomes	Brazil, *L. S. Kinoshita 02/101* (UEC)	DQ660774	DQ660584	DQ660705	DQ660519	DQ660646
Haplophyton crooksii (L. D. Benson) L. D. Benson	U.S.A., Arizona, *P. Jenkins 4901* (ARIZ)	DQ660775	DQ660585	DQ660706	DQ660520	DQ660647

Appendix 1. Continued.

Species	Voucher	GenBank accession numbers				
		rpl16 intron	*rps16* intron	3′ *trnK* intron	*matK*	*rbcL*
Hunteria eburnea Pichon	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S3029* (BR)	DQ660776	DQ660586	DQ660707	DQ660521	DQ660648
Kamettia chandeei D. J. Middleton	Thailand, *D. J. Middleton 3341* (E)	DQ660777	DQ660587	DQ660708	DQ660522	DQ660649
Kopsia fruticosa (Ker Gawl.) A. DC.	Thailand, *D. J. Middleton et al. 452* (A)	DQ660778	DQ660588	DQ660709	C	B
Lacmellea aculeata (Ducke) Monach.	French Guiana, *M. F. Prévost 3803* (CAY, P, Z)	DQ660779	DQ660589	DQ660710	DQ660523	DQ660650
Mascarenhasia arborescens A. DC.	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S3053* (R)	F	F	G	G	D
Melodinus australis (F. Muell.) Pierre	Australia, *P. I. Forster 27503* (BRI)	DQ660780	DQ660590	DQ660711	DQ660524	DQ660651
Melodinus cochinchinensis (Lour.) Merr.	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S3441* (BR)	DQ660781	DQ660591	DQ660712	DQ660525	DQ660652
Mesechites trifidus (Jacq.) Müll. Arg.	Ecuador, *Liede & Meve 3471* (UBT)	G	G	G	G	E
Molongum laxum (Benth.) Pichon	Venezuela, *P. Berry & E. Melgueiro 5400* (Z)	DQ660783	DQ660593	DQ660714	C	B
Neisosperma poweri (F. M. Bailey) Fosberg & M.-H. Sachet	Australia, *P. I. Forster 26594* (BRI)	DQ660784	DQ660594	DQ660715	DQ660527	DQ660654
Ochrosia elliptica Labill.	Australia, *P. I. Forster 27100* (BRI)	DQ660785	DQ660595	DQ660716	DQ660528	DQ660655
Odontadenia lutea (Vell.) Markgr.	Brazil, *L. S. Kinoshita 2005/56* (UEC)	F	F	G	G	DQ660656
Oncinotis tenuiloba Stapf	South Africa, *T. Abbott 7707* (PRU, Z)	DQ660786	DQ660596	DQ660717	DQ660529	DQ660657
Pachypodium lamerei Drake	cult., Switzerland, Bot. Gard. Zurich, *A. O. Simões 1333* (Z)	F	F	G	G	D
Parahancornia fasciculata (Poir.) Benoist	French Guiana, *M. F. Prévost 3801* (CAY, P, Z)	DQ660787	DQ660597	DQ660718	DQ660530	DQ660658
Parsonsia eucalyptophylla F. Muell.	Australia, *P. I. Forster 20118* (BRI)	DQ660788	DQ660598	DQ660719	DQ660531	DQ660659
Peltastes peltatus (Vell.) Woodson	Brazil, *M. P. Quast 9* (UEC); cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S3526* (BR)	DQ660789	DQ660599	DQ660720	DQ660532	E
Petopentia natalensis (Schltr.) Bullock	cult., U.S.A., Cornell, *Livshultz 03-2* (BH)	DQ660790	DQ660600	DQ660721	DQ660533	E
Phyllanthera grayi (P. I. Forst.) Venter	Australia, *P. I. Forster s.n.* (BRI)	DQ660791	DQ660601	DQ660722	DQ660534	DQ660660
Picralima nitida (Stapf) Th. & H. Dur.	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S3440* (BR)	DQ660792	DQ660602	DQ660723	C	B
Pleiocarpa mutica Benth.	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S1766* (BR)	DQ660793	DQ660603	DQ660724	DQ660535	E
Plumeria cubensis Urb.	cult., Hawaii, Nat. Trop. Bot. Gard. Kauai, Aug. 2003, *M. Endress s.n.* (NTBG)	DQ660794	DQ660604	DQ660725	DQ660536	DQ660661
Prestonia quinquangularis (Jacq.) Spreng.	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet S205* (BR)	DQ660795	DQ660605	DQ660726	C	B
Rauvolfia sellowii Müll. Arg.	Brazil, *I. Koch 868* (UEC)	DQ660796	DQ660606	DQ660727	DQ660537	DQ660662
Rauvolfia vomitoria Afzel.	cult., University of Campinas, *I. Koch 871* (UEC)	DQ660797	DQ660607	DQ660728	DQ660538	DQ660663
Saba comorensis (Bojer ex A. DC.) Pichon	cult., Belgium, Nat. Bot. Gard. Belgium, *F. Billiet 3142* (BR)	DQ660798	DQ660608	DQ660729	DQ660539	DQ660664
Schizozygia coffaeoides Baill.	cult., France, Bot. Gard. Chèvreloup, *acc. nr. 6331/613331* (unvouchered)	DQ660799	DQ660609	DQ660730	DQ660540	E
Secamone elliptica R. Br.	Thailand, *D. J. Middleton et al. 1162* (A)	DQ660800	DQ660610	DQ660731	DQ660541	DQ660665

Appendix 1. Continued.

Species	Voucher	GenBank accession numbers				
		rpl16 intron	*rps16* intron	3′ *trnK* intron	*matK*	*rbcL*
Stemmadenia litoralis (Kunth) L. Allorge	cult., Hawaii, Nat. Trop. Bot. Gard. Kauai, Aug. 2003, *M. Endress s.n.* (NTBG)	DQ660801	DQ660611	DQ660732	DQ660542	DQ660666
Stephanostema stenocarpum K. Schum.	Tanzania, *Luke 3754* (MO)	DQ660802	DQ660612	DQ660733	DQ660543	B
Strophanthus boivinii Baill.	cult., Switzerland, Bot. Gard. Zurich, Dec. 2003, *M. Endress s.n.* (Z)	DQ660803	DQ660613	DQ660734	DQ660544	DQ660667
Tabernaemontana amygdalifolia Jacq.	Mexico, *R. Ramirez & M. Garcia 527* (MEXU)	DQ660806	DQ660616	DQ660737	DQ660547	DQ660670
Tabernaemontana bufalina Lour.	Thailand, *D. J. Middleton 187* (A)	DQ660807	DQ660617	DQ660738	DQ660548	DQ660671
Tabernaemontana catharinensis A. DC.	Brazil, *A. O. Simões 1039* (UEC)	DQ660808	DQ660618	DQ660739	DQ660549	DQ660672
Tabernaemontana pachysiphon Stapf	cult., Brazil, Agronomic Institute of Campinas, *A. O. Simões 1325* (UEC)	DQ660809	DQ660619	DQ660740	DQ660550	DQ66006703
Telosma cordata (Burm. f.) Merr.	cult., U.S.A., Cornell, *T. Lirshultz 01-33* (BH)	DQ660810	DQ660620	DQ660741	DQ660551	DQ660674
Theretia peruviana (Pers.) K. Schum.	cult., U.S.A., Univ. Conn. campus, *T. Lirshultz 03-34* (A)	DQ660811	DQ660621		C	B
Tonduzia longifolia (A. DC.) Markgr.	Mexico, *Munn-Estrada 2165* (MEXU)	DQ660812	DQ660622	DQ660742	DQ660552	DQ660675
Trachelospermum jasminoides (Lindl.) Lem.	cult., U.S.A., Univ. Fl. campus, *G. Ionta 420* (FLAS)	F	F	G	G	DQ660676
Vinca minor L.	cult., Switzerland, Bot. Gard. Zurich, *A. O. Simões 1332* (Z)	DQ660813	DQ660623	DQ660743	DQ660553	E
Willughbeia angustifolia (Miq.) Markgr.	Brunei, *D. J. Middleton et al. 727* (A)	DQ660814	DQ660624	DQ660744	DQ660554	DQ660677
Wrightia arborea (Dennst.) Mabb.	Thailand, *D. J. Middleton 240* (A)	DQ660815	DQ660625	DQ660745	DQ660555	D
OUTGROUP						
Gelsemium sempervirens (L.) J. St.-Hil. (Gelsemiaceae)	cult., Switzerland, Bot. Gard. Zurich (plant died)	DQ660771	DQ660581		C	A
Gentiana verna L. (Gentianaceae)	Switzerland, *M. P. Quast 14* (Z)	DQ660772	DQ660582	DQ660703	C	DQ660644
Mitrasacme oasena Dunlop (Loganiaceae)	Australia, *P. I. Forster 26711* (BRI)	DQ660782	DQ660592	DQ660713	DQ660526	DQ660653
Strychnos lucida R. Br. (Loganiaceae)	Australia, *P. I. Forster 24078* (BRI)	DQ660804	DQ660614	DQ660735	DQ660545	DQ660668
Strychnos minor Dennst. (Loganiaceae)	Australia, *P. I. Forster 26993* (BRI)	DQ660805	DQ660615	DQ660736	DQ660546	DQ660669

Appendix 2. Morphological matrix for Apocynaceae and outgroups. ? = missing data. Polymorphic states are indicated by numbers in brackets.

	1 .	6 .	11 .	16 .	21 .	26 .	31 .	36 .	41 .
Adenium obesum	00111	01003	00100	10111	00021	11231	11021	20001	0
Allamanda schottii	00102	01012	10000	00011	01030	11212	21021	02100	0
Alstonia scholaris	00102	01003	00000	00011	00021	11222	21021	0{12}200	0
Alyxia reinwardtii	10102	01003	00000	00010	00021	00302	20021	14000	0
Ambelania acida	00100	01003	00000	10011	00002	00002	21021	00???	?
Amsonia elliptica	20101	01003	00000	00011	00021	11202	21021	00300	0
Amsonia tabernaemontana	20101	01003	00000	00011	00021	11202	21021	00300	0
Apocynum cannabinum	20100	01003	00100	10111	11121	11230	11121	2{05}001	0
Asclepias syriaca	20100	01110	00121	10111	33021	10230	11310	30001	0
Aspidosperma australe	00101	00003	01000	00010	00021	11212	21021	00300	0
Aspidosperma cylindrocarpon	00101	00003	01000	00010	00021	11212	21021	00300	0
Beaumontia grandiflora	10100	01012	00100	10111	01032	11230	11021	24001	0
Callichilia orientalis	00100	01012	00000	10011	01011	10102	20021	00400	0
Carissa macrocarpa	01100	00012	00000	00010	00002	00302	21021	00000	0
Carissa ovata	0?100	00003	00100	00010	00002	00302	21021	00000	0
Catharanthus roseus	30100	01003	00000	00011	01021	11202	21021	05400	0
Cerbera manghas	00101	01003	10000	01011	00021	00302	21021	01001	0
Cerbera venenifera	00101	01003	10000	01011	00021	00302	21021	01001	0
Chilocarpus costatus	10100	01003	00000	00010	00000	11102	20021	1????	?
Condylocarpon isthmicum	10102	01003	00000	00010	00021	01202	20120	?????	?
Couma guianensis	00102	01003	00000	00010	00100	00002	21021	0????	?
Craspidospermum verticillatum	00102	01003	00000	00011	00000	11212	21121	2?300	0
Diplorhynchus condylocarpon	00100	01003	00000	00011	00121	11212	21021	00200	0
Dyera costulata	00102	01012	00000	00011	00121	11212	21021	0????	?
Funtumia elastica	00100	01010	00100	10111	01021	11231	01021	20010	0
Geissospermum laeve	00101	00003	01100	00010	00021	00002	21021	0?200	0
Gonioma kamassi	00102	01003	00000	00010	00021	11212	21021	01300	0
Hancornia speciosa	00100	01003	00000	00010	00000	00002	21021	00???	?
Haplophyton crooksii	00100	01003	01000	00011	00021	11231	11021	0?300	0
Hunteria eburnea	00100	00012	00000	00010	00021	00002	21021	00300	0
Kamettia chandeei	10102	01003	00000	00010	00021	11212	21021	0????	?
Kopsia fruticosa	00100	01003	00100	00011	01021	00302	21021	02300	0
Lacmellea aculeata	00100	01003	00000	00010	00000	00002	21021	00???	?
Mascarenhasia arborescens	00100	01012	00100	10111	01021	11230	11021	200?0	0
Melodinus australis	10100	01003	00000	00010	00002	00002	21021	00300	0
Melodinus conchinchinensis	10100	01003	00000	00010	00002	00002	21021	00300	0
Mesechites trifidus	10100	01112	00100	10111	01021	11230	11021	2????	?
Molongum laxum	00100	01012	00000	10011	00000	00002	21021	00???	?
Neisosperma poweri	00102	01003	00100	00011	01021	00302	21021	01200	0
Ochrosia elliptica	00102	01003	00100	00011	01021	00302	21021	01200	0
Odontadenia lutea	10100	01010	00100	10111	01021	11230	11021	24???	?
Oncinotis tenuiloba	10100	00110	00100	10111	01121	11230	11021	20000	0
Pachypodium lamerei	00101	01003	00100	10111	01021	11230	11021	22001	0
Parahancornia fasciculata	00100	01003	00000	00010	00000	00002	21021	0????	?
Parsonsia eucalyptophylla	10110	01011	00100	10111	01032	11230	11021	22000	1

Appendix 2. Continued.

	1 .	6 .	11 .	16 .	21 .	26 .	31 .	36 .	41 .
Peltastes peltatus	10110	01011	00100	10111	01021	11230	11021	2????	?
Petopentia natalensis	10100	01010	00110	00111	22121	11230	11221	20001	0
Phyllanthera grayi	10100	01010	00110	00111	22121	11230	11221	2????	?
Picralima nitida	00100	00003	00000	00010	00021	00002	21021	00200	0
Pleiocarpa mutica	00100	00003	00000	00010	00021	00002	21021	00300	0
Plumeria cubensis	00101	01003	00000	00011	00121	11212	21021	02100	0
Prestonia quinquangularis	10110	01011	00100	10111	01021	11230	11021	25000	1
Rauvolfia sellowii	01102	01003	00000	00011	01012	00302	21021	00200	0
Rauvolfia vomitoria	01102	01003	00000	00011	01021	00302	21021	00200	0
Saba comorensis	10100	11003	00000	00010	00000	00002	21021	00???	?
Schizozygia coffaeoides	00100	01012	00100	10011	01021	11102	20021	00300	0
Secamone elliptica	10100	01010	00120	10111	34021	11230	11300	30001	0
Stemmadenia litoralis	00100	01012	00000	10011	01021	10102	20021	00400	0
Stephanostema stenocarpum	00100	01010	00000	10111	00021	11231	01021	20???	?
Strophanthus boivini	00100	01010	00100	10111	00112	11231	11021	21001	0
Tabernaemontana amygdalifolia	00100	01012	00000	10011	00021	10102	21021	00400	0
Tabernaemontana bufalina	00100	01012	00000	00010	00021	10102	21021	00400	0
Tabernaemontana catharinensis	00100	01012	00000	10011	00021	10102	21021	00400	0
Tabernaemontana pachysiphon	00100	01012	00000	10011	00021	10102	21021	00400	0
Telosma cordata	10100	01010	00121	10111	33021	10230	11310	30001	0
Thevetia peruviana	00101	01012	10000	01011	01021	00302	21021	01001	0
Tonduzia longifolia	00102	01010	00000	00011	01021	11222	21021	0?200	0
Trachelospermum jasminoides	10100	01012	00100	10111	01021	11230	11021	21???	?
Vinca minor	10100	01003	00000	00011	01021	11202	21021	00300	0
Willughbeia angustifolia	10100	10003	00000	00010	00000	00002	21021	0?000	0
Wrightia arborea	00100	01012	00000	10111	00032	11231	01021	20010	0
Gelsemium sempervirens	10020	01003	00300	02002	40032	11212	21021	06200	0
Gentiana verna	20020	00003	00100	02002	41030	11202	21021	0?100	0
Mitrasacme oasena	30020	00003	00200	01002	40002	11202	21021	000??	?
Strychnos lucida	00020	00003	00200	00002	40002	00002	21021	00200	0
Strychnos minor	10020	10003	00200	00002	40002	00002	21021	00200	0
	1 .	6 .	11 .	16 .	21 .	26 .	31 .	36 .	41 .

Appendix 3. Characters and character states for the morphological matrix used in the parsimony analyses.

1. **Habit**

Trees and shrubs (0); lianas or vines (1); perennial herbs (2); annual herbs (3).

The species of *Strophanthus* used in this analysis is an erect shrub; however, many species of this genus are large lianas.

2. **Sex expression**

Plants with perfect flowers (0); plants functionally dioecious or gynodioecious (1).

Functional dioecy has been reported in *Rauvolfia sellowii* and functional gynodioecy in *R. vomitoria* (Koch et al., 2002), coincidentally the two species of *Rauvolfia* chosen for this study. The only other genus of Apocynaceae in which functionally male or female flowers have been reported is *Carissa*, which also includes both functionally dioecious (e.g., *C. macrocarpa*, Correll & Correll, 1982; included in this study) as well as functionally gynodioecious (e.g., *C. grandiflora*, Marloth, 1932: 61; Koch et al., 2002) species. In functionally dioecious and gynodioecious species of both *Rauvolfia* and *Carissa*, the female plants have normal-appearing but sterile anthers, and the style is shorter than in the flowers of pollen-producing male plants.

3. **Non-articulated laticifers**

Absent (0); present (1).

Non-articulated laticifers are one of the key characters for the Apocynaceae s.l., distinguishing them from other families of the Gentianales.

4. **Latex color**

White, rarely yellow or orange (0); translucent (1).

Latex is typically white in Apocynaceae, but translucent latex is frequent within Echiteae, Wrightieae, and Malouetieae.

5. **Phyllotaxis**

Leaves opposite (0); leaves alternate (1); leaves mainly whorled, occasionally opposite at some nodes (2).

Opposite leaf arrangement is the usual condition in the family, with sporadic occurrence of other phyllotaxes. In the Alstonieae and Plumerieae, however, both alternate or whorled leaves are seen, and in Vinceae, whorled leaves are observed. Frequently in taxa with clearly whorled phyllotaxis, some nodes may have opposite leaves, especially in taxa with ternate phyllotaxis and in nodes closer to inflorescences. In such cases the taxon is coded as having a whorled phyllotaxis rather than being polymorphic.

6. **Tendrils**

Absent (0); present (1).

In Apocynaceae, hook-like tendrils are restricted to the Willughbeieae. The tendrils are modified inflorescences and may also bear flowers.

7. **Colleters in petiole axils**

Absent (0); present (1).

8. **Colleters adaxially at the base of the leaf blade**

Absent (0); present (1).

In Apocynaceae, colleters clustered or paired at the base of the leaf blade adaxially are common in the Asclepiadoideae and in some Secamonoideae and Periplocoideae. In Apocynaceae s. str., they are rare and characteristic for only two apocynoid clades: the Mesechiteae (Simões et al., 2004) and the close-knit trio of genera in Apocyneae subtribe Baisseinae sensu De Kruif (1983).

9. **Calycine colleters**

Absent (0); present (1).

In the Apocynaceae s. str., the absence or presence of calycine colleters is mostly consistent within a genus and is thus often used to distinguish genera. In addition, the distribution pattern of colleters within the calyx can be a useful tool to identify specimens and predict relationships (Woodson, 1933; Omino, 1996; Middleton, 1999; Endress & Bruyns, 2000; Simões & Kinoshita, 2002).

10. **Colleter arrangement within calyx**

One–15, in the sepal sinuses at the base (0); five, centered at the base sepal (1); 15 or more, more or less in a continuous ring, sometimes in more than one row (2).

11. **Infrastaminal appendages**

Absent (0); present (1).

Infrastaminal appendages, first described by Pichon (1948b), are outgrowths of the lower (congenitally fused) part of the corolla tube in the staminal sectors and are found in Plumerieae (e.g., *Thevetia*, *Cerbera*). The outgrowths in the congenitally fused part of the corolla tube in *Apocynum* are in the alternistaminal sectors and are therefore not considered to be homologous to infrastaminal appendages (Nilsson et al., 1993).

12. **Visible gaps in corolla tube**

Absent (0); present (1).

In Apocynaceae, the lower corolla tube (composed of the united petal and stamen primordia) is congenitally fused; the upper portion fuses postgenitally and basipetally, so that the last region to fuse is just above the insertion of the stamens. In a few genera (e.g., *Pleiocarpa*, *Picralima*, *Nerium*), flowers reach anthesis before fusion of the upper corolla is complete, leaving epidermal remnants or even visible gaps (Nishino, 1982, 1983; Erbar, 1991). Large gaps, visible with the naked eye or under a stereoscope, are characteristic for some Rauvolfioideae, e.g., *Aspidosperma* (Woodson, 1951), *Geissospermum* and *Haplophyton* (Fallen, 1986), and *Stephanostegia* (Leeuwenberg, 1997).

13. **Corolla lobe aestivation**

Sinistrorsely convolute (0); dextrorsely convolute (1); valvate (2); quincuncial imbricate (3).

Corolla lobe aestivation is one of the key taxonomic characters in the Apocynaceae. With a few exceptions, petals are almost always sinistrorsely convolute in the Rauvolfioideae, whereas in the other four subfamilies they are normally dextrorsely convolute or, in certain assemblages (e.g., Ceropegieae), valvate. The genera *Alstonia* (Sidiyasa, 1998), *Geissospermum* (Fallen, 1986; Allorge & Poupat, 1991), *Haplophyton* (Williams, 1995), and *Carissa* (Leeuwenberg & van Dilst, 2001) are rare in the family in that they contain both dextrorsely and sinistrorsely convolute species.

14. **Stamens**

Adnate to the corolla tube (0); adnate to five thickened basal corona lobes (1); adnate to a completely fused basal tube (2).

In Rauvolfioideae and Apocynoideae, stamens are always adnate to the corolla tube. In the other subfamilies, the stamens are more commonly inserted on coronas. In the Periplocoideae and Secamonoideae, this is in the form of a basal corona of five free to nearly fused lobes (Nilsson et al., 1993; Endress & Bruyns, 2000).

15. **Number of anther locules**

4 (0); 2 (1).

The anthers have four locules in all subfamilies of Apocynaceae except the Asclepiadoideae, in which there has been a loss of the two dorsal locules (Kunze, 1996).

16. **Lignified guide rails**

Absent (0); present (1).

Lignified guide rails are a specialization of the lateral parts of the anther and have a function in the complex pollination mechanism in Apocynaceae (Schick, 1982; Fallen, 1986; Kunze, 1991, 1996; Moré et al, 2007, this issue); they are absent in most Rauvolfioideae, but characteristic for Apocynoideae. Lignified guide rails are also characteristic for the majority of the genera of the Tabernaemontaneae but are absent, however, in species of *Tabernaemontana* section *Ervatamia* (see Leeuwenberg, 1994b: xv).

17. **Anther dehiscence**

Introrse (0); latrorse (1); extrorse (2).

18. **Gynostegium**

Absent (0); present (1).

The presence of a gynostegium (anthers postgenitally united with the style-head) is a key character in the family and distinguishes the subfamilies of the APSA clade (Livshultz et al., 2007) from the Rauvolfioideae (Leeuwenberg, 1994a; Endress & Bruyns, 2000).

19. **Style-head**

Absent (0); present (1).

Diagnostic within Apocynaceae, the style-head is the product of the postgenital fusion of the apical parts of the carpels and is enlarged and specialized, with zones covered by a secretory epithelium (Schick, 1980; Endress et al., 1983; Fallen, 1986).

20. **Style-head body differentiation**

Body of style-head with epidermis not clearly differentiated into regions specialized for pollen transport adhesive production and for receptive function (0); body of style-head with epidermis clearly differentiated into distinct regions specialized for the production of pollen transport adhesive or for receptive function (1).

Typically in Apocynaceae, the uppermost parts of the carpels do not fuse, resulting in two free apices at the top of the style-head. Schick (1980, 1982) demonstrated that these free appendages are sterile and that the receptive region of

the style-head is typically at the base, often beneath a membranous collar or wreath of longer hairs. In some Rauvolfioideae (e.g., *Picralima*, *Alyxia*), the style-head has a simple shape, usually ovoid or globular, without collar or wreath, and is not differentiated into distinct zones. In these genera, except for the sterile free apices, the entire surface of the style-head is covered with an epithelium that functions both as stigma and for the production of pollen transport adhesive (Schick, 1980; Endress et al., 1996).

21. **Pollen transport adhesive**

A foamy adhesive (0); five amorphous sticky translators via desiccation (1); five firm differentiated translators with spoon and viscidium (2); five firm differentiated translators with corpusculum and caudicles (3); absent (4).

Translators are composed of secretions of the style-head that harden through desiccation and form the apparatus for the transport of pollen. Complex, functionally differentiated translators are found in all Periplocoideae, Secamonoideae, and Asclepiadoideae (Kunze, 1993). Simple, functionally uniform translators have been reported in *Apocynum* and *Forsteronia* of Apocynoideae (Nilsson et al., 1993).

22. **Nectar-secreting tissue**

Absent or adnate and indistinct on outer wall of ovary or stipe (0); on lobes around ovary, these sometimes fused into an annulus (1); in the alternistaminal sectors on the sides of/between the basal corona lobes (2); in alternistaminal pockets on the basal tube (3).

Nectaries are absent in many Rauvolfioideae; they are present as lobes surrounding the base of the ovary in most Apocynoideae. In Periplocoideae, Secamonoideae, and Asclepiadoideae, nectar is secreted in alternistaminal pockets (Endress & Bruyns, 2000).

23. **Ovary position**

Superior (0); half-inferior (1); inferior (2).

24. **Ovary fusion**

Congenitally syncarpous (0); congenitally syncarpous to about halfway, apocarpous above (1); apocarpous (2); postgenitally syncarpous (3).

Postgenitally syncarpous ovaries are apocarpous in origin but fuse early during development. Postgenital syncarpy in Apocynaceae results most commonly in so-called double follicles, as found in *Parsonsia*, *Thenardia* Kunth, or *Temnadenia* Miers; the capsular fruit of *Allamanda* is also postgenitally syncarpous (Fallen, 1985).

25. **Placentation**

Parietal (0); marginal (1); axile (2).

26. **Fruit dehiscence**

Indehiscent (0); dehiscent (1).

27. **Pericarp consistency**

Fleshy (0); dry or woody (1).

28. **Seed presentation**

Embedded in pulp (0); with aril (1); naked (2); surrounded by a lignified or sclerified stone (3).

In most cases in which the seed is enclosed within a lignified or sclerified envelope, this is formed by the endocarp. The genera of the Carisseae present a special situation, in which the seed is surrounded by the lignified placentas, and thus not embedded in pulp, although the fruits of this tribe are fleshy berries (Pichon, 1952).

29. **Seed margin**

Naked (0); winged (1); fimbriate or ciliate (2); comose (3).

In *Craspidospermum*, the seed margin is surrounded by unusual-shaped, flattened appendages (Leeuwenberg, 1997: 14), which are here interpreted as a special kind of dissected wing and are not homologous with the fimbria or cilia found around the margin of the seed in, for example, *Alstonia* or *Tonduzia* (Sidiyasa, 1998, fig. 2.11). The coma is a terminal tuft of hairs on a seed and is thus not considered to be homologous to hairs, cilia, or fimbria surrounding the seed margin.

30. **Chalazal coma**

Absent (0); present (1).

Imprecise terminology, such as, "coma apical," with reference to a coma present at either end of the seed, has led to considerable confusion (see *Funtumia* in Sennblad et al., 1998). In the Apocynaceae, chalazal comas are on the end of the seed directed toward the base of the fruit, in contrast to the micropylar comas, which are on the end of the seed directed toward the apex of the fruit. The orientation of the coma hairs is irrelevant in determining whether a coma is chalazal or micropylar. For example, in *Kibatalia* G. Don, the seeds have a long-rostrate coma on the end of the seed directed toward the base of the fruit; the long hairs of the coma itself, however, are bent backward toward the fruit apex. Despite the direction of the hairs, *Kibatalia* is considered to have a basal coma due to its position on the seed in relation to the fruit. Due to difficulties in determining homology, chalazal and micropylar comas are coded as two separate characters. Chalazal comas are found only in the two tribes at the base of Apocynoideae: Wrightieae and Malouetieae. In some genera, only a chalazal coma is present, whereas in others, the seeds may have a coma at both the chalazal and micropylar ends, often with the micropylar one being the more robust and the chalazal one early deciduous. In other Apocynoideae and in all Periplocoideae, Secamonoideae, and Asclepiadoideae, the coma is exclusively micropylar, except for a few rare instances where it has been secondarily lost, usually in conjunction with a shift to water dispersal, e.g., several species of *Malouetia*, Apocynoideae (Woodson, 1933); *Finlaysonia obovata* Wall., Periplocoideae (Venter & Verhoeven, 2001); *Marsdenia rubrofusca* Benth. ex Fourn. and *Matelea amazonica* Morillo, Asclepiadoideae (Morillo, 1978, 1997).

31. **Micropylar coma**

Absent (0); present (1).

32. **Endosperm**

Ruminate (0); not ruminate (1).

Ruminate endosperm, though relatively rare in Apocynaceae, is characteristic for *Tabernaemontana* and is also found in most genera of the Alyxieae (Endress et al., 2007).

33. **Pollen dispersal unit**

Monads (0); tetrads without thinner inner walls (1); tetrads with thinner inner walls (2); pollinia (3).

In Rauvolfioideae and Apocynoideae, pollen is normally in monads. In Apocynoideae, only *Apocynum* has pollen in tetrads, whereas in Rauvolfioideae, tetrads occur sporadically in a few genera: *Callichilia*, *Condylocarpon*, *Craspidospermum*, and some *Melodinus* (Erdtman, 1952; Lienau et al., 1986). Tetrads are characteristic for the Periplocoideae, but these differ from those in Apocynoideae and Rauvolfioideae by their thinner inner walls (Nilsson et al., 1993).

34. **Sporopollenin layer covering pollinium**

Absent (0); present (1).

In Secamonoideae and a few genera of Periplocoideae, all tetrads of a theca form a pollinium. The pollinia in these taxa differ from those in the Asclepiadoideae in that they lack an outer sporopollenin layer (Verhoeven & Venter, 2001).

35. **Pollen apertures**

Absent (0); present (1).

In Asclepiadoideae and Secamonoideae, pollen grains within the pollinia are inaperturate. Pollen is aperturate in all other subfamilies with the exception of *Condylocarpon* (Rauvolfioideae, Alyxieae), which has inaperturate, nearly exineless pollen grains that remain in tetrads (van der Ham et al., 2001).

36. **Aperture type**

Colporate (0); porate with large (6–22 μm), irregularly disposed and ± unequal-sized pores (1); porate with small (2–5 μm), regularly disposed and equal-sized pores (2).

Apocynoideae, Periplocoideae, Secamonoideae, and *Craspidospermum* (Melodineae) are characterized by having porate pollen grains. In Rauvolfioideae, in contrast, pollen grains are almost always colporate (Nilsson, 1986). Within Rauvolfioideae, several genera of the Alyxieae, however, have porate pollen; this pollen differs from the porate pollen in the other subfamilies in that the pores are of varying sizes, often very large (up to 86 μm), and are not arranged in a regular pattern on the grain (van der Ham et al., 2001).

37. **Chromosome number**

$x = 11$ (0); $x = 10$ (1); $x = 9$ (2); $x = 6$ (3); $x = 12$ (4); $x = 8$ (5); $x = 4$ (6).

38. **Secoiridoids and complex indole alkaloids**

Absent (0); secoiridoids present, indole alkaloids absent (1); characteristic indole alkaloids present, those with secologanin skeleton non re-arranged (2); characteristic indole alkaloids present, including those with re-arranged secologanin skeleton of the eburnan and/or plumeran type (3); characteristic indole alkaloids present, including those with re-arranged secologanin part of the ibogan type (4).

An estimated 1600 different structural representatives of alkaloids have been isolated in Apocynaceae, which are classified by their biogenetic relationship. Of these, complex indole alkaloids are restricted to Rauvolfioideae and are categorized by their skeletal type (Kisakürek et al., 1983). Here we used a slightly simplified version of this system, adapted for our taxon sampling.

39. **Steroidal alkaloids**

Absent (0); present (1).

Steroidal alkaloids do not occur in Rauvolfioideae. They seem to be most common in the Malouetieae of the Apocynoideae (Endress et al., 1990; Bisset, 1992).

40. **Cardenolides**

Absent (0); present (1).

Cardenolides are found sporadically in various parts of the family. In Rauvolfioideae, they are found, for example, in *Acokanthera* (Carisseae), *Thevetia*, and *Cerbera* (Plumerieae) (Hegnauer, 1970, 1989).

41. **Pyrrolizidine alkaloids**

Absent (0); present (1).

Pyrrolizidine alkaloids have been reported from various genera of Apocynoideae, particularly *Parsonsia* and *Prestonia* of the tribe Echiteae (Brehm et al., 2007, this issue).

Appendix 4. Revised tribal classification of Rauvolfioideae. Genera transferred to tribes different from those in the classification of Endress and Bruyns (2000) are indicated in bold. A description of each tribe is provided.

RAUVOLFIOIDEAE Kostel., Allg. Med.-Pharm. Fl. 3: 1054. 1834. TYPE: *Rauvolfia* L., Sp. Pl. 1: 208. 1753.

ASPIDOSPERMEAE Miers, Apocyn. S. Amer. 7. 1878. TYPE: *Aspidosperma* Mart. & Zucc., Flora 7 (Beil. 4): 135. 1824, nom. cons.

Trees or shrubs. Latex usually milky, but sometimes red or yellow in *Aspidosperma*. Leaves alternate or opposite, rarely whorled. Calyx 5-, rarely 4-, 6-, or 7-parted, calycine colleters absent; corolla salverform or somewhat funnelform in *Haplophyton*, with slits in the tube behind the stamens, corolla lobe aestivation sinistrorsely convolute (dextrorsely convolute in *Geissospermum* and one species of *Haplophyton*); corona absent; style-head body mostly undifferentiated, concomitantly receptive and secretory, with a short basal collar in *Haplophyton*; ovary apocarpous; disc absent. Fruit normally with dry pericarp and dehiscent, a pair of follicles, or sometimes one aborted, but fruit fleshy in *Geissospermum* and *Vallesia*. Seeds various: thin and compressed with papery wing in *Aspidosperma* and *Strempeliopsis*, or not compressed and with coma at both ends (*Haplophyton*), or naked (*Microplumeria*, *Vallesia*). Pollen 3-colporate. Secondary chemistry indole alkaloids.

Aspidosperma Mart. & Zucc., Flora 7 (Beil. 4): 135. 1824. nom. cons.

Geissospermum Allemão, Pl. Nov. Bras. 707. 1846.

Haplophyton A. DC., Prodr. 8: 412. 1841.

Microplumeria Baill., Bull. Mens. Soc. Linn. Paris 1: 749. 1889.

Strempeliopsis Benth. in Benth. & Hook. f., Gen. Pl. 2: 702. 1876.

Vallesia Ruiz & Pav., Fl. Peruv. Chil. Prodr. 28. 1794.

ALSTONIEAE G. Don, Gen. Hist. 4: 70, 86. 1838. TYPE: *Alstonia* R. Br., Asclepiadeae 64. 1810, nom. cons.

Trees or shrubs with milky latex. Leaves whorled or opposite. Flowers mostly small, salverform to tubular (rotate in *Dyera*); calycine colleters absent except in *Dyera*; corolla lobe aestivation either sinistrorse or dextrorse; corona absent; anthers normally ovate, unspecialized (connective with an apical extension in *Dyera*); style-head usually without a basal collar; ovary apocarpous, or partially syncarpous at the base and apocarpous above (completely syncarpous in *Alstonia rostrata*) and more than half inferior in *Dyera*; disc absent, adnate, or inconspicuous, (some species of *Alstonia* with a distinct annular disc). Fruit with dry pericarp and dehiscent, a pair of follicles or these rarely fused; ovules numerous per carpel. Seeds thin, compressed, testa covered with long hairs (*Alstonia*) or surrounded by a papery wing (*Dyera*). Pollen 3-colporate. Secondary chemistry indole alkaloids.

Alstonia R. Br., Asclepiadeae 64. 1810, nom. cons.

Dyera Hook. f., J. Linn. Soc. Bot. 19: 293. 1882.

VINCEAE Duby, Bot. Gall. 1: 324. 1828. TYPE: *Vinca* L., Sp. Pl. 1: 209. 1753.

Trees or shrubs, more rarely lianas, vines or herbs, with milky latex. Leaves whorled or opposite, rarely alternate. Calycine colleters absent; corolla salverform (infundibuliform in *Vinca*); free corona lobes absent, but the orifice sometimes with a thickened ring; corolla lobe aestivation usually sinistrorse (dextrorse in *Kopsia* and *Ochrosia*); stamens inserted at the middle of the corolla tube or above, included; anthers ovate, without lignified basal appendages, unspecialized (except in *Vinca*), filaments almost always short (except in *Vinca*); style-head usually with the stigmatic region beneath a basal (sometimes membranous) collar; ovary apocarpous (in some *Rauvolfia* species hemi-syncarpous), usually glabrous, often only one carpel maturing; disc normally present. Fruit composed of 2 separate mericarps (sometimes only one developing), these either indehiscent, drupaceous, with a fleshy pericarp and stony endocarp, or with a pair of thin-walled follicles; seeds 1 to 4(to 6) per carpel, rarely more, ovoid or compressed, mostly not winged (the flat margins forming a narrow rim in *Kopsia* and *Ochrosia*); in *Tonduzia* and *Laxoplumeria* seeds numerous, elliptic-compressed with long cilia around the margin; endocarp mostly forming a stone. Pollen mostly 3-colporate. Secondary compounds indole alkaloids.

Catharanthus G. Don, Gen. Hist. 4(1): 95. 1837.

Kamettia Kostel., Allg. Med.-Pharm. Fl. 3: 1062. 1831.

Kopsia Blume, Cat. Gew. Buitenzorg (Blume) 12. 1823, nom. cons.

Laxoplumeria Markgr., Notizbl. Bot. Gart. Berlin-Dahlem 9: 981. 1926.
Ochrosia Juss., Gen. Pl. 144. 1789.
Petchia Livera, Ann. Roy. Bot. Gard. (Peradeniya) 10: 140. 1926.
Rauvolfia L., Sp. Pl. 1: 208. 1753.
Tonduzia Pittier, Contr. U.S. Natl. Herb. 12: 103. 1908.
Vinca L., Sp. Pl. 1: 209. 1753.

WILLUGHBEIEAE A. DC., Prodr. 8: 318. 1844. TYPE: *Willughbeia* Roxb., Pl. Coromandel 3: 77, t. 280. 1819, nom. cons.

Trees, shrubs, or woody lianas, the last often with grappling tendrils, with milky latex. Leaves opposite (verticillate in *Couma*); calycine colleters present or absent; corolla salverform; corolline corona absent; corolla lobe aestivation almost always sinistrorse; stamens included; apical connective appendages of anthers often scarcely developed; style-head usually without basal collar, sometimes body uniformly receptive; ovary congenitally syncarpous; disc mostly absent, when present adnate or indistinct from ovary. Fruit indehiscent, a berry with fleshy, nonfibrous pericarp and placentas becoming pulpy in fruit; seeds ovoid, embedded in pulp, with or without a longitudinal furrow; endosperm smooth or wrinkled longitudinally. Pollen (1 to)3(to 4)-colporate. Secondary chemistry indole alkaloids.

Ancylobotrys Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 91. 1898.
Bousigonia Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 35. 1898.
Chamaeclitandra (Stapf) Pichon, Mém. Inst. Franç. Afrique Noire 35: 202. 1953.
Clitandra Benth. in Hook., Niger Fl. 445. 1849.
Couma Aubl., Hist. Pl. Guiane Suppl. 39, t. 392. 1775.
Cyclocotyla Stapf, Bull. Misc. Inform. 1908: 259. 1908.
Cylindropsis Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 38. 1898.
Dictyophleba Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 92. 1898.
Hancornia Gomes, Mem. Math. Phis. Acad. Real Sci. Lisboa 3: 51. 1812.
Lacmellea H. Karst., Linnaea 28: 449. 1857.
Landolphia P. Beauv., Fl. Oware Bénin 1: 54. 1801, nom. cons.
Leuconotis Jack, Trans. Linn. Soc. 14: 121. 1823.
Orthopichonia H. Huber, Kew Bull. 15: 437. 1962.
Pacouria Aubl., Hist. Pl. Guiane 268, t. 105. 1775.
Parahancornia Ducke, Arch. Jard. Bot. Rio de Janeiro 3: 242. 1922.
Saba (Pichon) Pichon, Mém. Inst. Franç. Afrique Noire 35: 302. 1953.
Vahadenia Stapf in Thiselton-Dyer, Fl. Trop. Africa 4(1): 29. 1902.
Willughbeia Roxb., Pl. Coromandel 3: 77, t. 280. 1819, nom. cons.

TABERNAEMONTANEAE G. Don, Gen. Hist. 4: 70, 87. 1838. TYPE: *Tabernaemontana* L., Sp. Pl. 1: 210. 1753.

Trees or shrubs, rarely lianas, with milky latex. Leaves opposite, sometimes anisophyllous. Inflorescences axillary, terminal, or (usually paired) in the forks of branches; calycine colleters mostly present and centered on the lower part of the sepal, often multiseriate, rarely absent, very rarely (*Tabernanthe*) alternisepalous; corolla usually salverform, less often tubular-campanulate or infundibuliform; corona absent; corolla lobe aestivation almost always sinistrorse (dextrorse in *Schizozygia*, 1 species of *Callichilia*, and 2 species of *Tabernaemontana*); stamens mostly sessile or nearly so, anthers mostly with lignified guide rails (these absent in some species of *Tabernaemontana*); style-head with the stigmatic region beneath a basal flange and usually with an (often 5-ribbed) upper crest or the style-head subglobose and without a basal collar and upper crest and body uniformly receptive; ovary syncarpous or apocarpous; disc surrounding the base of the ovary, adnate, indistinct, or free. Fruit with fleshy pericarp, usually an indehiscent berry with the seeds embedded in pulp or pair of dehiscent follicles with arillate seeds; seed testa often wrinkled, pitted, or with longitudinal ridges, often with a long hilar groove. Pollen 3- to 5-colporate, sometimes zonocolporate; in *Callichilia* in tetrads. Secondary chemistry highly evolved indole alkaloids of the heynean type.

Ambelania Aubl., Hist. Pl. Guiane 265, t. 104. 1775.
Bonafousia A. DC., Prodr. 8: 359. 1844.
Callichilia Stapf in Thiselton-Dyer, Fl. Trop. Afr. 4(1): 130. 1902.
Calocrater K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 175. 1895.
Carvalhoa K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 189. 1895.
Crioceras Pierre, Bull. Mens. Soc. Linn. Paris 1: 1311. 1897.
Macoubea Aubl., Hist. Pl. Guiane Suppl. 17, t. 378. 1775.
Molongum Pichon, Mém. Mus. Natl. Hist. Nat. 24: 167. 1948.
Mucoa Zarucchi, Agric. Univ. Wageningen Pap. 87(1): 40. 1988.
Neocouma Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 33. 1898.
Rhigospira Miers, Apocyn. S. Am. 67, t. 10A. 1878.
Schizozygia Baill., Bull. Mens. Soc. Linn. Paris 1: 752. 1888.
Spongiosperma Zarucchi, Agric. Univ. Wageningen Pap. 87(1): 48. 1988.
Stemmadenia Benth., Bot. Voyage Sulphur 124, t. 44. 1845.
Stenosolen (Müll. Arg.) Markgr. in Pulle, Fl. Suriname 4(1): 455. 1937.
Tabernaemontana L., Sp. Pl. 1: 210. 1753.
Tabernanthe Baill., Bull. Mens. Soc. Linn. Paris 1: 782. 1888.
Voacanga Thouars, Gen. Nov. Madag. 10. 1806.
Woytkowskia Woodson, Ann. Missouri Bot. Gard. 47: 74. 1960.

MELODINEAE G. Don, Gen. Hist. 4: 71, 101. 1838. TYPE: *Melodinus* J. R. Forst. & G. Forst., Char. Gen. Pl.: 37, t. 19. 1776.

Trees or shrubs; latex milky. Leaves opposite or whorled. Calycine colleters usually absent; corolla salverform, sometimes unfused (with gaps) just above the insertion of the stamens; corolla lobe aestivation sinistrorse; small corolline corona often present in petal sinuses (well developed and usually annular and petaloid in *Melodinus*); stamens included, anther connective with both apical connective extension and small sterile basal lobes in *Pycnobotrya*; style-head mostly without basal collar or upper wreath; ovary apocarpous or congenitally syncarpous; disc absent or an adnate, indistinct annulus around the base of the ovary. Fruit mostly a pair of follicles with dry pericarp (fruit a capsule in *Craspidospermum* and a fleshy berry in *Melodinus*); seeds usually numerous, smooth, flat and winged, often peltately attached with a long funicle (somewhat verrucose, only slightly compressed and embedded in pulpy placenta in *Melodinus*). Pollen usually 3-colporate (in tetrads in some *Melodinus* species, and porate tetrads in *Craspidospermum*). Secondary compounds indole alkaloids.

Craspidospermum Bojer ex A. DC., Prodr. 8: 323. 1844.
Melodinus J. R. Forst. & G. Forst., Char. Gen. Pl.: 37, t. 19. 1776.
Pycnobotrya Benth. in Benth. & Hook. f., Gen. Pl. 2: 715. 1876.
Stephanostegia Baill., Bull. Mens. Soc. Linn. Paris 1: 748. 1888.

ALYXIEAE G. Don, Gen. Syst. 4: 70, 96. 1838. TYPE: *Alyxia* Banks ex R. Br., Prodr. 469. 1810, nom. cons.

Trees, shrubs, or vines, with milky latex. Leaves often whorled, sometimes opposite, rarely alternate. Calycine colleters absent; corolla salverform (sometimes throat somewhat expanded in *Condylocarpon*); corolla lobes inflexed in bud or not; aestivation sinistrorse in bud; corona absent; stamens inserted in the upper half of the corolla tube, included; filaments short; anthers ovate, unspecialized; style-head without basal collar or upper wreath, body uniformly receptive; ovary apocarpous or syncarpous, normally bicarpellate with axile or marginal placentation (up to 5-carpellate in *Lepinia* and *Lepiniopsis*, and in *Chilocarpus* placentation parietal), often stipitate; disc absent, indistinct, or adnate. Fruit usually indehiscent (dehiscent in *Chilocarpus* and *Plectaneia*), with fleshy pericarp and stony endocarp (pericarp dry in *Condylocarpon*, *Chilocarpus*, and *Plectaneia*); seeds usually globular or ovoid, with deep hilar groove or depression (seeds arillate in *Chilocarpus* and compressed with wings in *Plectaneia*). Pollen 2- to 3-porate (mostly barrel-shaped with two pores, but in tetrads in *Condylocarpon*). Neither alkaloids nor cardenolides are known to occur in the tribe.

Alyxia Banks ex R. Br., Prodr. 469. 1810, nom. cons.
Chilocarpus Blume, Cat. Gew. Buitenzorg (Blume) 22. 1823.
Condylocarpon Desf., Mém. Mus. Nat. 8: 119. 1822.
Lepinia Decne., Ann. Sci. Nat., Bot. sér. 3, 12: 194, t. 9. 1849.
Lepiniopsis Valeton, Ann. Jard. Bot. Buitenzorg 12: 251. 1895.
Plectaneia Thouars, Gen. Nov. Madag. 11. 1806.
Pteralyxia K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 151. 1895.

HUNTERIEAE Miers, Apocyn. S. Amer. 6. 1878. TYPE: *Hunteria* Roxb., Fl. Indica 2: 531. 1824.

Trees or shrubs, rarely lianas, with milky latex. Leaves opposite, rarely in whorls of 3. Calycine colleters mostly present and centered at the base of the sepal, often multiseriate, rarely absent; corolla salverform; aestivation sinistrorse; corona absent; anthers ovate, without lignified guide rails, sometimes shortly sagittate at the base, included; style-head ovoid to narrowly clavate, without basal collar or upper crest, but often with slender, elongate non-receptive apices; ovary apocarpous, 2- to 5-carpellate, glabrous; disc an adnate or indistinct annulus around the base of the ovary. Fruit indehiscent, composed of 2 to 5 baccate mericarps with fleshy, fibrous pericarp; seeds ovoid, without longitudinal furrow, embedded in pulp. Pollen 3-colporate. Secondary chemistry indole alkaloids.

Gonioma E. Mey., Comment. Pl. Africae Austr. 188. 1838.
Hunteria Roxb., Fl. Indica 2: 531. 1824.
Picralima Pierre, Bull. Mens. Soc. Linn. Paris 2: 1278. 1896.
Pleiocarpa Benth. in Benth. & Hook. f., Gen. Pl. 2: 699. 1876.

PLUMERIEAE E. Mey., Comm. Pl. Africae Austr. 2: 188. 1838. TYPE: *Plumeria* L., Sp. Pl. 1: 209. 1753.

Trees or shrubs, the latter sometimes scrambling, with milky latex. Leaves usually alternate, sometimes opposite (whorled in *Allamanda*). Calycine colleters absent or present; corolla salverform, infundibuliform, or tubular-campanulate; corolline corona often present below the petal sinuses behind stamen (usually much dissected in *Allamanda*) and infrastaminal appendages generally present; aestivation sinistrorse; anther connective often broadened, resulting in sublaterse dehiscence and with an elongate apical extension, these often exserted, anthers otherwise included; style-head mostly with basal collar or lobes (no distinct basal collar in *Plumeria*, *Himatanthus*, and *Mortoniella*), mostly without upper wreath (well-developed upper wreath present in *Allamanda*), but often with free apices conspicuously enlarged; ovary apocarpous or syncarpous at the very base (postgenitally syncarpous in *Allamanda*), hemi-inferior in *Plumeria*, *Himatanthus*, and *Mortoniella*; disc absent or present. Fruit either indehiscent, a pair of drupes or samaroids with fleshy or dry pericarp or dehiscent and a pair of follicles (in *Allamanda* fruit a capsule, usually with spines); seeds 1 to 4(to many) per carpel, usually with compressed winglike margin or distinct papery wing. Pollen 3-colporate. Secondary compounds cardenolides or iridoid glycosides (in *Skytanthus*, *Skytanthus* alkaloids).

Allamanda L., Mant. 146: 214. 1771.
Anechites Griseb., Fl. Brit. W.I. (Grisebach) 410. 1861.
Cameraria L., Sp. Pl. 1: 210. 1753.
Cerbera L., Sp. Pl. 1: 208. 1753.
Cerberiopsis Vieill. ex Pancher & Sébert in Sébert, Not. Bois. Nov. Caledonie 187. 1874.
Himatanthus Willd. ex Schult. in Roem. & Schult., Syst. Veg. 5: 221. 1819.
Mortoniella Woodson, Ann. Missouri Bot. Gard. 26: 257. 1939.
Plumeria L., Sp. Pl. 1: 209. 1753.
Skytanthus Meyen, Reise 1: 376. 1834.
Thevetia L., Opera Varia 212. 1758, nom. cons.

CARISSEAE Dumort., Anal. Fam. Pl. 26. 1829. TYPE: *Carissa* L., Mant. 52. 1767, nom. cons.

Shrubs or small trees, rarely scandent, with or without (branched) spines in leaf axils, with milky latex. Leaves opposite, less frequently verticillate. Inflorescences usually axillary, cymose, mostly very contracted, ± umbelliform; calycine colleters almost always absent; corolla salverform, usually white or cream, sometimes flushed with pink, the lobes not inflexed in bud; aestivation sinistrorse or dextrorse; corona absent; stamens inserted at the middle of the corolla tube or above; anthers free from the style-head, ovate, unspecialized; style-head scarcely differentiated, without basal collar, body uniformly receptive; ovary congenitally syncarpous, bilocular, usually glabrous; placentation axile; disc absent or an indistinct annulus adnate to the base of the ovary. Fruit indehiscent, a berry with fleshy, non-fibrous, non-sclerotic pericarp; endocarp not stony, but placentas becoming indurated in fruit forming pseudo-stone; seeds 2 to 6(to 12), rarely more, compressed, without longitudinal furrow. Pollen 3-colporate. Secondary chemistry cardenolides.

Acokanthera G. Don, Gen. Hist. 4(2): 485. 1838.
Carissa L., Mant. 52. 1767, nom. cons.

INCERTAE SEDIS WITHIN RAUVOLFIOIDEAE

Amsonia Walter, Fl. Carol. 98. 1788.

Amsonia has long been considered related to taxa belonging to Vinceae sensu Endress and Bruyns (2000). Although in our analysis it is clearly unrelated to Vinceae, its affinities are uncertain. A sister relationship to Alyxieae is suggested, but this is only moderately supported and without substantiating morphological evidence.

Diplorhynchus Welw. ex Ficalho & Hiern, Trans. Linn. Soc. London, Bot. ser. 2, 2: 22, t. 5. 1881.

Diplorhynchus was included in the Melodineae sensu Endress and Bruyns (2000). Due to the unresolved placement of this genus in our analysis, we feel it is most prudent to place it as incertae sedis until more data become available.

A PHYLOGENETIC ANALYSIS OF THE *CASCABELA–THEVETIA* SPECIES COMPLEX (PLUMERIEAE, APOCYNACEAE) BASED ON MORPHOLOGY[1]

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Abstract

A parsimony cladistic analysis based on 55 morphological characters was performed for the *Cascabela* Raf.–*Thevetia* L. species complex (Apocynaceae), including 22 terminals, sampling at least one species of each genus in Plumerieae sensu Endress and Bruyns. The consensus of the 12 most parsimonious trees (length = 164, consistency index = 0.50, retention index = 0.75) can be correlated to three previous subtribal classifications. The topology supports the monophyly of the *Cascabela–Thevetia* species complex, but it is not conclusive to whether *Cascabela* should be recognized as a genus or as a subgenus of *Thevetia*. Because there are two important and easy-to-diagnose characters supporting each clade (digitiform suprastaminal appendages and embryos not compressed in *Cascabela*; reniform fruits and segmented endocarp in *Thevetia*), the recognition of two genera is preferred here. Therefore, the new combination *C. pinifolia* (Standl. & Steyerm.) Alvarado-Cárdenas & Ochot.-Booth is proposed. Keys and synoptic descriptions for the two genera and their species are provided.

Key words: Apocynaceae, *Cascabela*, *Cerbera*, Cerbereae, morphology, Plumerieae, *Thevetia*, Thevetiinae.

The Apocynaceae s.l. family have approximately 5000 species and 395 genera classified in five subfamilies (Endress & Bruyns, 2000; Endress, 2004), of which Rauvolfioideae is the most diverse and heterogeneous, with 10 tribes. Among these tribes, the circumscription of Plumerieae has changed according to several authors (Table 1), and Endress and Bruyns (2000) proposed the most recent tribal classification, based on morphology and recent DNA phylogenetic hypotheses. According to this classification, Plumerieae consists of 11 genera (Endress & Bruyns, 2000; Sennblad & Bremer, 2002) including *Cascabela* Raf., *Cerbera* L., and *Thevetia* L. These genera share a close morphological relationship, which is reflected in the recognition by some authors (Lippold, 1980; Alvarado-Cárdenas, 2003) of a generic complex with an intricate taxonomic history. This begins with Tournefort (1700), who first applied the name *Ahouai* Tourn. for *T. ahouai* (L.) A. DC. Later, in the works *Genera Plantarum* and *Hortus Cliffortianus*, and *Opera Varia* Linnaeus (1737a, 1737b, 1758, respectively) changed *Ahouai* to *Thevetia*, in honor of the French monk A. Thevet. However, in subsequent editions of *Species Plantarum* (Linnaeus, 1753) and *Genera Plantarum* (Linnaeus, 1754), Linnaeus included *Thevetia* within *Cerbera*, starting the confusion regarding the generic circumscription and creating disagreements among subsequent authors. Throughout the history of this generic complex, several names are involved, including *Ahouai* Mill. (Miller, 1751) and *Thevetia* Adans. (Adanson, 1763), and later *Plumeriopsis* Rusby & Woodson (Woodson, 1937) and *Ahovai* Pichon (Pichon, 1948a), but none of these names were correctly applied, as discussed in the taxonomic revision of *Thevetia* by Lippold (1980). After this work, the complex was divided into three genera, based on fruit characters and flower differences (Fig. 1), but Lippold's position is still in question. The long controversy includes two principal proposals: (1) to recognize *Thevetia* as different from *Cerbera*, but not from *Cascabela* (Gensel, 1969; Williams, 1996a, b; Allorge, 1998; Morales, 2005); and (2) to recognize three generic entities: *Cascabela*, *Cerbera*, and *Thevetia* (Rafinesque-Schmaltz, 1838; Lippold, 1980; Gentry, 1998; Potgieter & Albert, 2001; Alvarado-Cárdenas,

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Table 1. Different proposals of classification for the taxa that were classified within Rauvolfioideae, Plumerieae by Endress and Bruyns (2000). In all the proposals, *Cascabela* is considered as a synonym of *Thevetia*. NI = not included.

	Authors					
Genera	Endlicher (1838)	de Candolle (1844)	Schumann (1897)	Pichon (1948a, b, 1950a)	Leeuwenberg (1983, 1994)	Sennblad & Bremer (2002)
Allamanda L.	Subordo-1 Carisseae	Willughbeieae Allamandeae	Plumerioideae Arduineae Landolphiineae	Plumerioideae Allamandeae	Plumerioideae Allamandeae	Rauvolfioideae Plumerieae
Anechites Griseb.	NI	NI	Plumerioideae Plumerieae Rauwolfiineae	Plumerioideae Rauvolfieae Anechitinae	Plumerioideae Rauvolfieae Anechitinae	Rauvolfioideae Plumerieae
Cameraria L.	NI	Plumerieae Tabernaemontanineae	Plumerioideae Plumerieae Alstoniineae	Cerbereoideae Thevetieae Camerariinae	Plumerioideae Cerbereae	Rauvolfioideae Plumerieae
Cascabela Raf.	Subordo-2 Ophioxyleae	Carissaeae Thevetieae	Plumerioideae Plumerieae Cerberiineae	Cerbereoideae Thevetieae Thevetiinae	Plumerioideae Cerbereae	NI
Cerbera L.	Subordo-2 Ophioxyleae	Plumerieae Tabernaemontanineae	Plumerioideae Plumerieae Cerberiineae	Cerbereoideae Thevetieae Cerberinae	Plumerioideae Cerbereae	Rauvolfioideae Plumerieae
Cerberiopsis Vieill. ex Pancher & Sebert	NI	NI	Plumerioideae Plumerieae Cerberiineae	Cerbereoideae Thevetieae Cerberinae	Plumerioideae Cerbereae	NI
Himatanthus Willd. ex Roem. & Schult.	NI	NI	NI	Plumerioideae Plumeriinae	Plumerioideae Plumeriinae	NI
Mortoniella Woodson	NI	NI	NI	Plumerioideae Plumeriinae	Plumerioideae Plumeriinae	NI
Plumeria L.	Subordo-3 Euapocyneae Plumerieae	Plumerieae Tabernaemontanineae	Plumerioideae Plumerieae Alstoniineae	Plumerioideae Plumeriinae	Plumerioideae Plumeriinae	Rauvolfioideae Plumerieae
Skytanthus Meyen	NI	Echiteae	Plumerioideae Plumerieae Plumerieae Alstoniineae	Cerbereoideae Skytantheae Skytantheae	Plumerioideae Cerbereae Cerbereae	NI
Thevetia L.	Subordo-2 Ophioxyleae	Carissaeae Thevetieae	Plumerioideae Plumerieae Cerberiineae	Cerbereoideae Thevetieae Thevetiinae	Plumerioideae Cerbereae	Rauvolfioideae Plumerieae

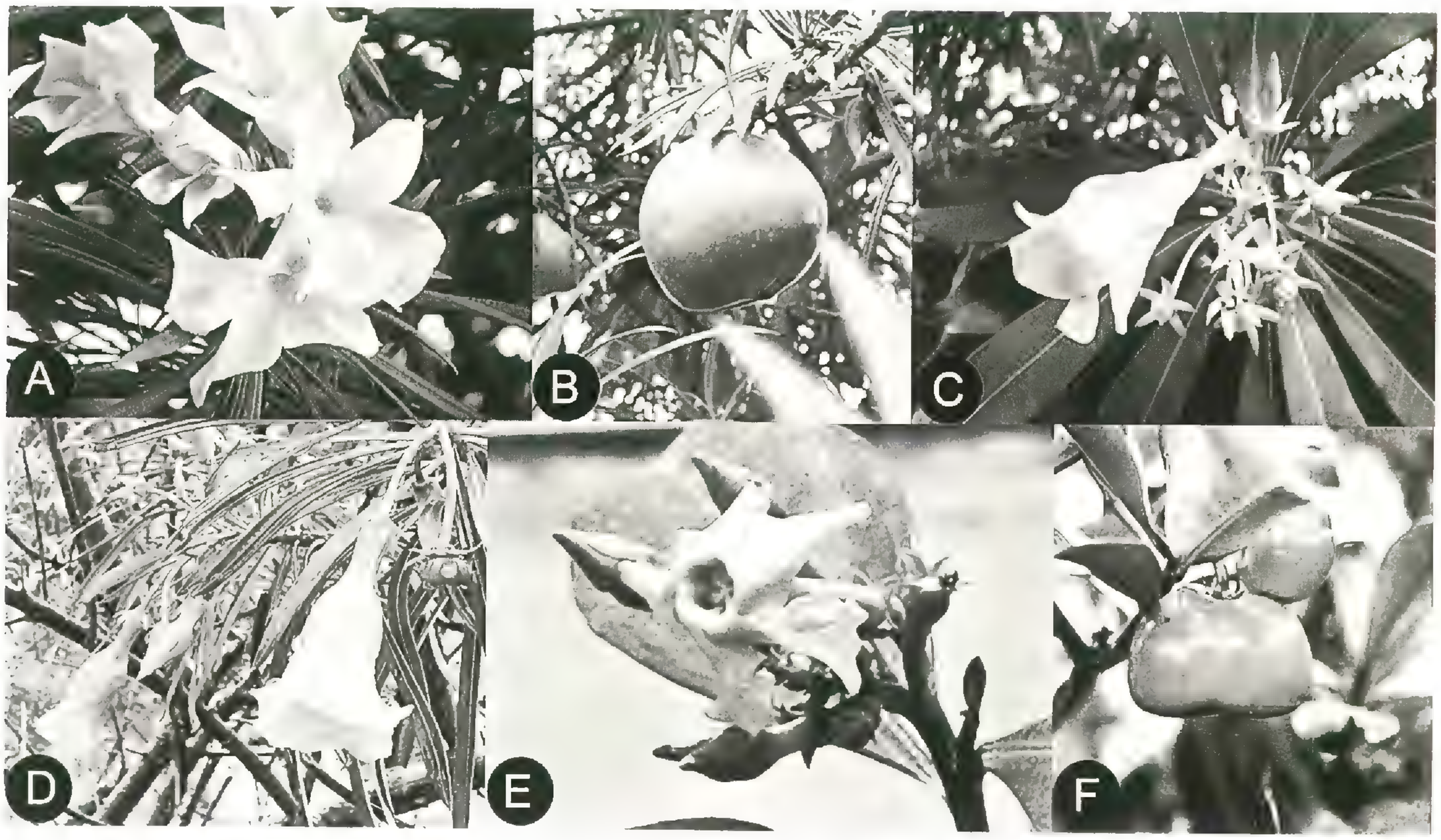

Figure 1. Exemplar species belonging to the *Cascabela–Thevetia* complex. —A, B. *Cascabela thevetioides*. —C. *C. gaumeri*. —D. *C. thevetia*. —E, F. *Thevetia ahouai*.

2003; Alvarado-Cárdenas & Ochoterena, 2005). Not recognizing *Thevetia* as a different genus from *Cascabela*, Williams (1996a) elevated *T. peruviana* K. Schum. in Engl. & Prantl var. *pinifolia* Standl. & Steyerm. ex Leavenw. (Leavenworth, 1946) to species rank. Therefore, eight species are currently recognized within *Thevetia* (including *Cascabela*), with six within *Cerbera*. Despite the recent cladistic analyses to classify the family and the tribe based on morphology and/or molecular data (Endress et al., 1996; Sennblad & Bremer, 1996, 2002), these works have not included all the species in the complex; they instead focused on broader questions, leaving aside the problematic circumscription of *Cascabela* and *Thevetia*.

The objective of this work is to provide evidence based on a morphological phylogenetic study for a more natural classification of the generic complex. Also, the intention is to postulate hypotheses for the relationships among the species within the species complex and to test the position of the genera within the tribe. At the same time, the results of this analysis will allow for the postulation of hypotheses for the evolution of flower and fruit characters related to pollination and dispersal syndromes. A key and synoptic descriptions of the taxa in the complex are provided to facilitate future taxonomic work.

Material and Methods

An intensive bibliographic compilation (monographic and floristic treatments, phylogenetic studies, etc.) was assembled to define the species sampling, as well as to compare the observations and coding of the morphological character set: Allorge (1998); Alvarado-Cárdenas (2003, 2004); Boiteau & Allorge (1978); Endress (1996); Endress & Bruyns (2000); Endress et al. (1983, 1996); Ezcurra (1981); Fallen (1983, 1984, 1985, 1986); Gensel (1969); Gentry (1998); Leeuwenberg (1983, 1994, 1999); Nilsson (1986, 1990); Nowicke (1970); Pichon (1948a, b, 1949, 1950a, b); Pire (1989); Plumel (1991); Potgieter & Albert (2001); Rosatti (1989); Roubik & Moreno (1991); Rzedowski & Rzedowski (1998); Sennblad & Bremer (1996, 2002); Sennblad et al. (1998); Simoes & Kinoshita (2002); Standley (1924); Standley & Williams (1958); Veillon (1971); Williams (1996a, b, 2002); Woodson (1935, 1938a, b); and Woodson & Moore (1938).

Taxon Sampling

Twelve genera and 22 species were included in the cladistic analysis (Table 2). This sampling includes for the first time all species of *Cascabela* (four species) and *Thevetia* (four species), three representative species of *Cerbera* (out of six), and at least one species of each genus within Plumerieae sensu Endress and Bruyns (2000). *Carissa macrocarpa* (Eckl.) A. DC. was used as the functional outgroup according to Sennblad and Bremer (1996), who suggested that it is a member of the sister tribe Carissae (*Carissa* L. and *Acokanthera* G. Don f.). This outgroup was also selected considering potential

Table 2. List of genera and species included in the analysis.

Genera	Species total/included	Species
Carissa	30/1	*C. macrocarpa* A. DC.
Allamanda	14/1	*A. cathartica* L.
Anechites	1/1	*A. nerium* (Aubl.) Urb.
Camercria	3/1	*C. latifolia* L.
Cascabela	4/4	*C. gaumeri* (Hemsl.) Lippold
		C. ovata (Cav.) Lippold
		C. thevetia (L.) Lippold
		C. thevetioides (Kunth) Lippold
Cerbera	6/3	*C. floribunda* K. Schum.
		C. odollam Gaertn.
		C. manghas L.
Cerberiopsis	3/1	*C. candelabra* Vieill. ex Pancher & Sebert
Himatanthus	13/1	*H. obovatus* (Müll. Arg.) Woodson
Mortoniella	1/1	*M. pittieri* Woodson
Plumeria	7/2	*P. obtusa* L.
		P. rubra L.
Skytanthus	2/2	*S. acutus* Meyen
		S. hancorniaefolius (A. DC.) Miers
Thevetia	4/4	*T. ahouai* (L.) A. DC.
		T. amazonica Ducke
		T. bicornuta Müll. Arg.
		T. pinifolia (Standl. & Steyerm.) J. K. Williams

inclusive synapomophies, as proposed by Nixon and Carpenter (1994).

CHARACTER SAMPLING

The homology hypotheses represented by the character and character state definitions follow the conjunction and similarity (position, form, and function) criteria of Patterson (1982) and De Pinna (1991). Floral and vegetative macromorphological structures were studied from personal collections and more than 900 sheets from herbarium material listed in Appendices 1 and 2. The specimens are deposited in the following herbaria: G, MEXU, MO, NY, SP, XAL, and Z. Reproductive characters were studied from the spirit material and flower anatomical section slide collections of M. Endress at Z.

Pollen grains and floral structures observed under a scanning electron microscope (SEM; Hitachi S-2460N) at the Institute of Biology, UNAM were dehydrated and covered with gold-palladium inside an ionized camera (Emitech K550). Pollen grains for observation under optic microscopy were acetolyzed following Erdtman (1960) and mounted on slides using glycerin. At least 20 grains per species were measured at polar and equatorial axis lengths. The pollen and floral structures were sampled with the authorization of their respective herbaria (MEXU, MO, NY). Sampled collections are indicated by a dagger (†) in Appendix 2.

The following continuous characters were analyzed using descriptive statistics to define the character states: bract length (7), sepal length (9), and pollen diameter (22). Species-level box graphs were constructed from all of the specimen measurements, considering standard error and deviation, using the program Statistica V.6.0.3 (not shown, available from the authors; Statsoft Corporation, Tulsa, Oklahoma). The intervals, which correspond to the proposed character states, were defined to reduce overlap of standard deviation.

PHYLOGENETIC ANALYSIS

A morphological matrix (Table 3) was constructed and edited in WinClada (Nixon, 2002), and analyzed using NONA (Goloboff, 1999). To find the most parsimonious trees (MPTs), heuristic searches using tree bisection-reconnection (TBR) were conducted with 2000 replications (in sets of 1000) using different starting trees (Wagner trees built with random addition sequences), holding 20 trees on each replication. The searches were followed by a more extensive TBR holding up to 50,000 trees (twice: h50,000; h/20; mu*1000; max*; sv*). Branches with ambiguous support in the MPTs were collapsed, identical trees were removed, and a consensus tree was calculated using the option "Consensus (strict)" in WinClada and saved as a metafile edited in Adobe Photoshop 6.0. The option "apo[" of NONA (Goloboff,

Table 3. Morphological matrix used in the analysis. Characters and character states as in Appendix 3. Ambiguity key: inapplicable = -; missing data = ?; polymorphism A = 0, 1, B = 1, 2. Generic key: A = *Allamanda*, An = *Anechites*, C = *Cameraria*, Ca = *Carissa*, Cas = *Cascabela*, Ce = *Cerbera*, Cerb = *Cerberiopsis*, H = *Himatanthus*, M = *Mortoniella*, P = *Plumeria*, S = *Skytanthus*, T = *Theretia*.

	0 5 10 15 20 25 30 35 40 45 50
Ca. macrocarpa	111010-0010100-1100000000110-1000200-0000220200-0-0--30
P. rubra	20210110101000-1100000000010-0-2120102000011O---0012020
P. obtusa	20210110101000-1100000000010-0-2120102000011O---0012020
H. obovatus	20210112000000-1100000000010-0-2120102000011O---0010030
M. pittieri	20100110000000-0100000000010-0-2120100000010O---0011030
T. cathartica	122100-0020021001101001000112O-1021102010210 30--0010000
S. acutus	100A110A010010-00-1210000001 0-2020202010111O---01 --1[illegible]
S. hancornaefolius	10BA0100000001010-1210000001 0-2020211010111O---0011030
An. nerium	011100-0001100-1101110100000-1020A12110100100---010-030
C. latifolia	1110010000000 1010-1210111001011201121101 -10310-6113030
Cerb. candelabra	20210111110001001111111010011O-2010211011-10301C011303O
Ce. odollam	2020011B120011111111112C100110-2C1C211C1020?3211011[illegible]0
Ce. floribunda	20200111110001111111111B0100110-2010211010201321101100O0
Ce. manghas	20200112120001011111112O100110-2010211010221321101100 0
I. tacorcida	102110-1010121011111111011011113001211110320?110110--??
I. amazonica	100110-1010101011111111011011113001211110 3?111101 0--11
T. pinifolia	B0011101010121211111A111110121230012111102312 0-0.13132
T. ahouai	B0211A0001010101111111211101111300121111032011101110131
Cas. ovata	2021010101012121111111111101212300121111023122[illegible]0113132
Cas. gaumeri	2020110101012121111111111101212300121111023022[illegible]-0113132
Cas. thevetioides	2001010101012121111A[illegible]1212300121111023122O-0113132
Cas. theretia	2000110A0101212111111111101212300121111023122O-0113132

1999) was used to identify the character states that unambiguously support the branches of the consensus in all MPTs. With this option, the potential artifacts that result from mapping characters onto the collapsed branches of a consensus (Nixon & Carpenter, 1996) are corrected. To evaluate the relative stability of clades in the consensus, a character removal analysis (Davis et al., 1993), as applied in Luna and Ochoterena (2004), was conducted using NONA (Goloboff, 1999) enabled through the corresponding option in WinClada (Nixon, 2002), using the same parameters as for the original matrix. The goal of this analysis was also to evaluate the contribution of individual characters to the topology of the consensus in order to postulate strategies for future research.

Results and Discussion

A total of 55 potentially informative characters were included in the matrix (Table 3); 22 are multistate and six of them (1, 7, 9, 22, 33, 54) were coded as additive (cf. Appendix 3). The matrix has a total of 1210 cells, with eight (0.6%) of them coded as missing (?) and 75 (6.1%) as inapplicable (-).

The heuristic search yielded 12 MPTs (length (L) = 164, consistency index for entire tree (CI) = 0.50, retention index (RI) = 0.75), the consensus of which (Fig. 2; L = 174, CI = 0.47, RI = 0.72) recovers two main clades. The main hierarchical groups correlate to the three taxa proposed by Pichon (1948b, 1950a) and Leeuwenberg (1983, 1994): (1) Plumeriinae, represented here by *Himatanthus* Willd. ex Roem. & Schult., *Mortoniella* Woodson, and *Plumeria* L.; (2) Allamandeae, represented by *Allamanda* L., and (3) Cerbereae, represented by the remaining genera. Based on the proposal of Endress and Bruyns (2000), we suggest adjusting these proposed groups to the rank of subtribes, which by nomenclatural priority would be Plumeriinae, Allamandinae, and Thevetiinae (Table 1; Fig. 2).

Allamanda

The position of *Allamanda* as sister to Thevetiinae (supported by shape of the upper extension of the connective [character 19/state 1, cf. Appendix 3; consistency index for character (ci) = 0.66], presence of infrastaminal appendages [27/1, ci = 0.50], and receptivity pattern type 1 [39/1, ci = 1.0]) conflicts with the hypotheses based on molecular data (Sennblad & Bremer, 1996) and combined analyses (Endress et al., 1996; Potgieter & Albert, 2001). In those analyses, species of *Allamanda* form the most inclusive clade, sharing a most recent common ancestor with *Himatanthus* and *Plumeria*. These conflicting results reflect the morphological complexity of *Allamanda* and its 14 species, and reinforce the need to continue investigating its phylogenetic position. Provisionally, our results support the recognition of a separate subtribal category for *Allamanda* (Allamandinae) as previously suggested (Pichon, 1948b; Leeuwenberg, 1983, 1994).

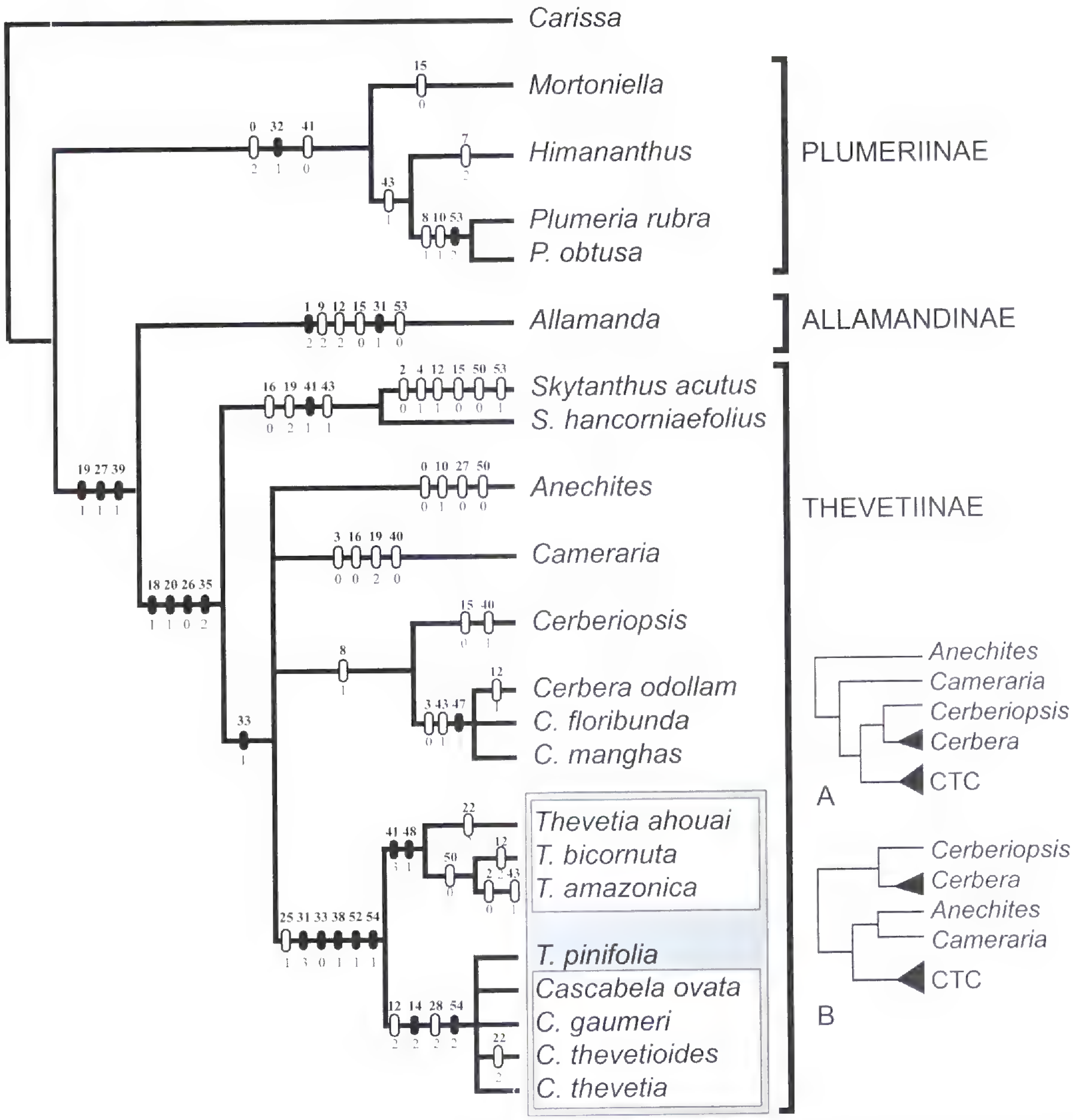

Figure 2. Consensus of 12 most parsimonious trees (MPTs, L = 164, CI = 0.50, RI = 0.75) for the members of tribe Plumerieae and the suggested subtribal classification. Solid ellipses represent synapomorphic states and hollow ones homoplasies. Only nonambiguous character states present in all MPTs are mapped onto the consensus. Numbers above the ellipses correspond to the characters and the numbers below them to the states. Inside the gray rectangle are the species classified by Gensel (1969) as *Thevetia* subg. *Thevetia* and *Thevetia* subg. *Yccotli* (*Cascabela*). Inside the white rectangles are the species considered by Lippold (1980) as belonging to *Thevetia* and *Cascabela*. A, B. Topologies found among the MPTs showing the two alternative hypotheses for the sister group of the *Cascabela–Thevetia* clade (CTC). —A. Topology that supports the traditional view. —B. Topology that supports, in part, the molecular works.

RELATIONSHIPS WITHIN SUBTRIBE PLUMERIINAE

The consensus tree supports the monophyly of Plumeriinae, which corroborates previous classifications by Pichon (1948b, 1950a), Leeuwenberg (1983, 1994), and Plumel (1991), who suggested a close relationship among *Himatanthus*, *Mortoniella*, and *Plumeria*. Plumeriinae is supported as monophyletic by these characters: life form (0/2, ci = 0.50); ovary position (32/1, ci = 1.0); and shape of non-compressed fruits (41/0, ci = 0.75). Our results also corroborate Potgieter and Albert (2001), where *Plumeria* and *Himatanthus* are also sister.

RELATIONSHIPS WITHIN SUBTRIBE THEVETIINAE

Thevetiinae is supported as monophyletic by having the anther dehiscence latrorse (18/1, ci = 1.0), a dark apical connective color (20/1, ci = 1.0), absence of a depression in the mesocolpium (26/0, ci = 1.0), and

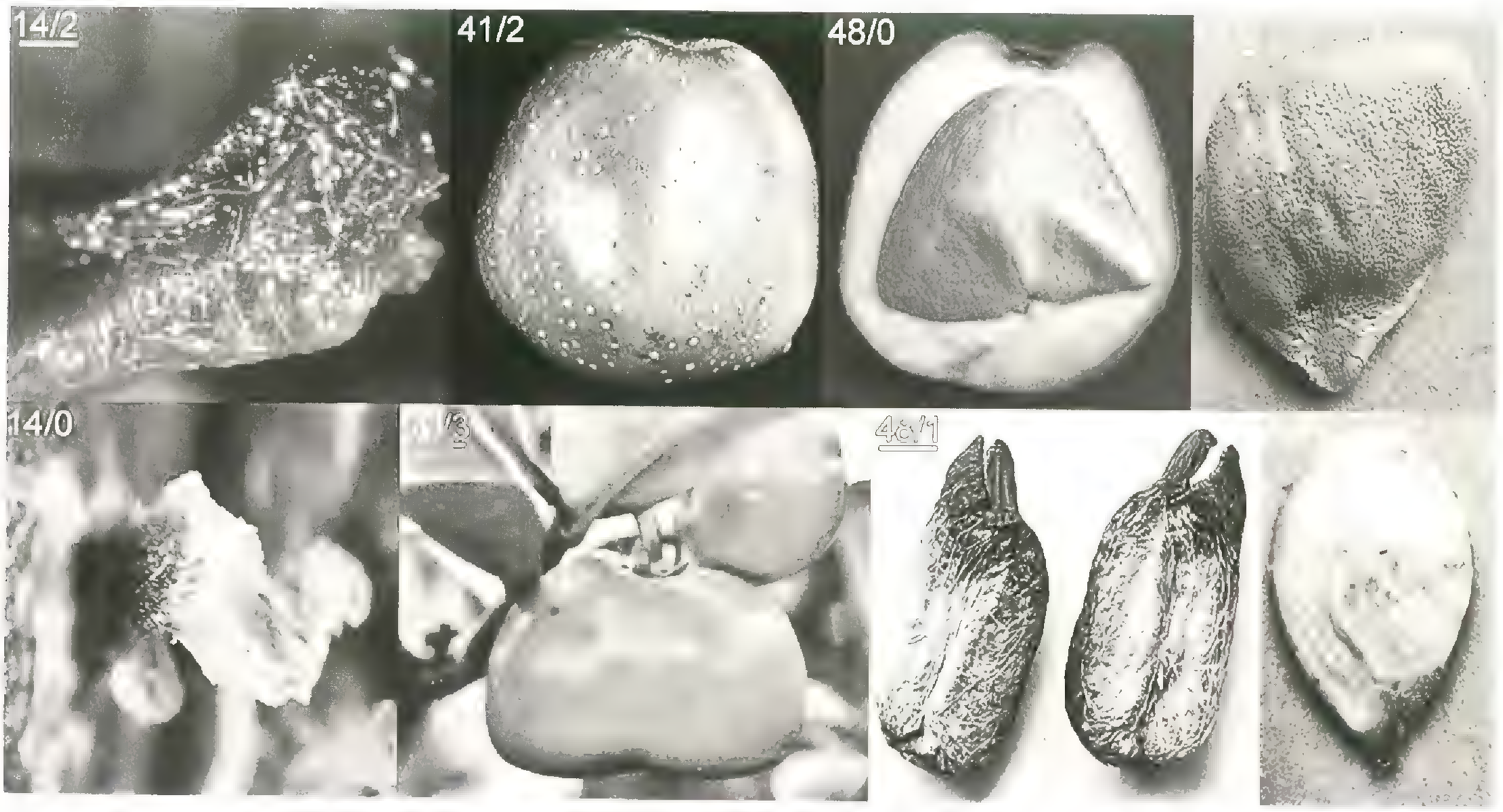

Figure 3. Characters/character states (see Appendix 3) that support the monophyly of *Cascabela* (upper row) and *Thevetia* (lower row). The underlined numbers indicate synapomorphic states that support the corresponding genera.

conical stigmata (35/2, ci = 1.0). These characters were previously used to circumscribe the elements of the subfamily Cerberioideae (Pichon, 1948a; Fallen, 1983).

Skytanthus Meyen was resolved as monophyletic, supported by the combination of sessile anthers (16/0, ci = 0.50), a filamentous upper extension of the connective (19/2, ci = 0.66), fusiform fruits (41/1, ci = 0.75), and lenticels on the exocarp (43/1, ci = 0.20). The position of this genus as the earliest derived within the subtribe is somewhat in agreement with Pichon (1948a), who suggested the genus as the most primitive within his subfamily Cerberioideae.

Phylogenetic relationships of the Cascabela–Thevetia *species complex.* Within subtribe Thevetiinae, three clades correspond more or less to *Cascabela*, *Cerbera*, and *Thevetia*, but *Cascabela* is resolved as paraphyletic, because *T. pinifolia* (Standl. & Steyerm.) J. K. Williams is nested with four other species of *Cascabela*. There is still no consensus regarding the question of whether one should recognize one genus with two subgenera (Gensel, 1969) or two distinct genera (Lippold, 1980), given that *Cascabela* and *Thevetia* are sister taxa (Fig. 2). Nevertheless, based on the unique combination and the clear contrast of character states (Fig. 3), we support the recognition of two genera: *Cascabela*, with two synapomorphies (digitiform suprastaminal appendages [14/2, ci = 0.6]; embryos not compressed [54/2, ci = 1.0]), and *Thevetia*, also with two synapomorphies (reniform fruits [41/3, ci = 0.75]; segmented endocarp [48/1, ci = 1.0]). Thus, we propose a new combination for *T. pinifolia* as *Cascabela* (see Appendix 4).

The relationship of *Cascabela* and *Thevetia* as sister taxa is in disagreement with the phylogeny presented by Potgieter and Albert (2001), where *T. ahouai* shared a most recent common ancestor with *Pteralyxia kauaiensis* Caum, while *T. peruviana* (Pers.) K. Schum. (= *Cascabela thevetia* (L.) Lippold) is sister to *Amsonia* Walter. Nevertheless, in our analysis, the sister relationship of these genera is strongly supported by five synapomorphies: partial syncarpy (31/3, ci = 1.0), two ovules per carpel (33/0, ci = 1.0), a secretory region cylindrical to elliptic (38/1, ci = 1.0), denticulate margins of the seed wing (52/1, ci = 1.0), and the embryo compressed on one side (54/1, ci = 1.0); in addition to one homoplasic character state, the presence of endofissures in the nexine (25/1, ci = 0.50). The discrepancy in the results by Potgieter and Albert (2001) could be explained by sampling artifacts (with only two representatives from the generic complex). Conversely, due to lack of material, our study did not include species of *Pteralyxia* K. Schum. & Prantl and *Amsonia*, and therefore future analyses should take into account these sampling issues.

Cerbera was resolved as monophyletic, supported by the absence of indumentum on vegetative parts (3/0, ci = 0.16), lenticels on exocarp (43/1, ci = 0.20), and the endocarp forming a network with the mesocarp (47/1, ci = 1.0). In the consensus (Fig. 2), this genus shares a most recent common ancestor with *Cerberiopsis* Vieill. ex Pancher & Sebert, supported by

having obovate sepals (8/1, ci = 0.50). The relationship of *Cerbera* as sister to *Cerberiopsis* restricts the generic complex to *Cascabela* and *Thevetia*. At the same time, this sister relationship contrasts with Pichon's (1948a) proposal to group *Cerberiopsis* and *Cameraria* L. within Cameriinae based on fruit compression. However, in our analysis this character appears in all MPTs twice independently (fruit strongly compressed in cross-section; 40/1, ci = 0.50). Our analysis corroborates Potgieter and Albert (2001), where *Cerbera* and *Cerberiopsis* are also sister taxa, as well as the observations of Leeuwenberg (1999) and Veillon (1971), who suggested a close relation between these genera based on vegetative and floral appearances.

The sister group of the *Cascabela–Thevetia* clade (hereafter named CTC) is uncertain due to the existence of two conflicting topologies among the MPTs that result in a polytomy in the consensus. Among the MPTs, one topology suggests a sister relationship of the CTC with *Cerbera–Cerberiopsis* (Fig. 2A), supported by the unique combination of obovate leaf blades (2/2, ci = 0.33), floral bracts 4–11.5 mm (7/1, ci = 0.40), and a connate apical connective (21/1, ci = 1.0). This resolution supports the traditional perception of Gensel (1969), Lippold (1980), and Alvarado-Cárdenas (2003). A second topology resolves *Cameraria* and *Anechites* Griseb. as sister to the CTC (Fig. 2B) and is supported by ornamentation on the infrastaminal trichomes (29/1, ci = 0.33); nectary (34/1, ci = 0.33); and a sclerified endocarp (45/1, ci = 0.50). These characters have never been employed to suggest relationship among genera within the tribe. Although this topology is in disagreement with tradition, it corroborates, in part, the relationship supported by molecular data (Sennblad & Bremer, 2002), which also recovered a similar clade including the four genera. According to Sennblad and Bremer (2002), *Anechites* is resolved as sister to *Thevetia*, with *Cerbera* as sister to them and *Cameraria* sharing their most recent common ancestor.

The resolution among species of *Cascabela* lacks hierarchic structure in the consensus (Fig. 2). Among the MPTs, there are three alternative topologies for their relationships, where *C. gaumeri* (Hemsl.) Lippold and *C. ovata* (Cav.) Lippold have the most variable position, placed either as the earliest or most derived species.

Character removal: Topological consensus resolution. Twenty-seven (5, 10–12, 13, 15, 17, 18, 20, 22, 23, 25, 26, 28, 31, 35–38, 42, 46, 47, 49, 51–54) out of the 55 characters can be removed without affecting the topology of the consensus.

Removing characters 3 (presence of indumentum on vegetative parts) or 4 (secondary veins) reduces conflict within the *Cascabela* clade. The topology obtained without any of these characters is the same as one of the alternative hypotheses among the original MPTs. By removing character 14 (shape of suprastaminal appendages), there is an increase in resolution within the *Cerbera* clade. When removing characters 1 (phyllotaxis), 9 (sepal length), or 45 (endocarp texture), the topology of the consensus trees supports the sister relationship of *Cerbera–Cerberiopsis* and the CTC. This topology coincides with one of the alternative resolutions among the original MPTs (Fig. 2A). In contrast, when characters 7 (length of bracts), 21 (apical connective arrangement), 24 (exine thickness), 30 (infrastaminal trichomes ornamentation pattern), or 44 (mesocarp consistency) are removed, the sister relationship of the clade *Cameraria–Anechites* and the CTC is recovered (Fig. 2B). Removing characters 41 (shape of non-compressed fruits) and 48 (endocarp presentation) results in lack of support for the genus *Thevetia* as monophyletic. Nevertheless, the reniform shape of the fruits and the segmented endocarp characteristic of all species of *Thevetia* present a strong homology hypothesis supported by morphological and anatomical studies (Pichon, 1948a, 1950b; Alvarado-Cárdenas, 2003). Therefore, we are convinced of the monophyletic nature of this genus. The removal of characters 6 (presence of a dominant axis in the inflorescences that branch) or 8 (sepal shape) results in a decrease in the resolution of the consensus within the *Thevetia* clade, and the clade *Cerbera–Cerberiopsis* is collapsed.

By removing characters 0 (life form), 27 (presence of infrastaminal appendages), 32 (ovary position), 39 (receptivity pattern), or 40 (fruit shape in cross-section), the relative position of *Mortoniella* and *Allamanda* is lost, collapsing both genera to a basal polytomy. The removal of characters 2 (leaf blade shape), 16 (presence of a structure supporting the anther), 29 (presence of ornamentation on the infrastaminal trichomes), 33 (number of ovules per carpel), 34 (presence of nectary), 43 (presence of lenticels on the exocarp), or 50 (presence of a wing on the seed) results in a decrease of resolution within subtribe Cerberineae. Some of these characters are postulated as homologies with the support of careful morphological studies (e.g., character 16 (Fallen, 1986); characters 33 and 50 (Pichon, 1948a)); others should be re-evaluated considering a bigger sample size and/or alternative coding (characters 2, 29, 41, 43). The removal of character 19 (shape of the upper extension of the connective) collapses the backbone of the consensus, but the clades corresponding to the CTC, *Cerbera–Cerberiopsis*, and Plumeriinae are still

supported. This character has been used before in phylogenetic analyses (e.g., Endress et al., 1996; Williams, 2002), but here, as mentioned, we highlight its vital importance to recover the topologies of the MPTs. Because this character does not include polymorphism within taxa and its states are unambiguously defined, we recommend that future analyses that consider morphology include it to test if it is corroborated by independent evidence.

EVOLUTION OF CHARACTERS RELATED TO REPRODUCTIVE BIOLOGY AND FLORAL MORPHOLOGY

For Apocynaceae subfamily Asclepiadoideae, numerous works related to pollination biology have been published. These works report mainly species of Diptera, Hymenoptera, and Lepidoptera involved in the complex systems of pollination. In contrast, this type of information for early derived groups within the family is scarce (Lopes & Machado, 1999). For Plumerieae, there are only records for *Himatanthus* and *Plumeria*, which are pollinated by sphingids displaying a process named "pollination by deceit" (Haber, 1984; Plumel, 1991; Knudsen & Tollsten, 1993; Oliveira et al., 2004). The species of *Himatanthus* and *Plumeria* lack nectaries, but they deceive pollinators by displaying many white flowers with strong and attractive fragrances produced at the same time that other species with nectar are in flower. *Mortoniella pittieri* Woodson probably has the same pollination system, because it shares the most recent common ancestor (Fig. 2) and the same character states (Table 3) with both genera. Several species from the *Cerbera–Cerberiopsis* clade (hereafter referred as the CCC) also have white flowers, have strong fragrances, and lack nectaries (Leeuwenberg, 1999). Given this pollination syndrome, it is possible that they are also pollinated by sphingids, but the only evidence to suggest that they also exhibit deceit pollination is the lack of nectaries. In contrast to the flower morphology of Plumerieae, the flowers of the CCC have suprastaminal and infrastaminal appendices, which may restrict the pollinators from reaching the pollen. The presence of these appendages (13/1, ci = 0.33) is a derived condition in all the MPTs, although the relative time of appearance is ambiguous.

Also with white flowers, *Anechites* and *Cameraria* differ from the previous genera in that they have nectaries. These structures allow us to predict that in the pollination system of both genera, there is no deceit pollination. It could be that all members of the tribe are pollinated by Lepidoptera, even those with yellow flowers (Weberling, 1989; Proctor et al., 1996) such as *Allamanda*, *Cascabela*, *Skytanthus*, or *Thevetia*; nevertheless, labels of herbarium material indicate that some species are visited by hummingbirds (*Cascabela ovata*: *Pérez A. 861*, MEXU) and bees (*C. ovata*: *Lott 430*, MEXU). Of all the genera with yellow flowers, only *Skytanthus* lacks nectaries. The interpretation of the evolution of nectaries (34) is ambiguous among the MPTs; in all cases, their presence is a derived condition, but in some hypotheses, their absence is plesiomorphic, whereas in others there is a secondary loss (ci = 0.5–0.33). It would be interesting to solve this ambiguity, because it may imply a relative increase in the complexity of the flowers, from flowers without nectaries or staminal appendages to flowers without nectaries but with staminal appendages to flowers with both structures.

An increase of structural complexity was suggested by Fallen (1986). She considered *Thevetia* (including *Cascabela*) to be one of the most derived genera within Plumerieae, due to the presence of a lobulate secretory region to which the infrastaminal appendages adhere, forming the gynostegium. Here we can corroborate this hypothesis. Not only is the presence of staminal appendages (13/1) derived, but so is the presence of a lobulate secretory region (38/1), which appears a posteriori as a synapomorphy of one of the most derived clades, *Cascabela–Thevetia*.

Endress (1996) suggested that the presence of appendages in the apical connective region of the anthers could be related to the pollination systems in subfamily Asclepiadioideae. Within Plumerieae, all the genera present these structures, but their shape is variable among genera. In our analysis, the obtuse shape of the anther apical connective (19/0) represents the plesiomorphic condition, which derived into the deltoid connective (19/1) that, in turn, transformed into filamentous connectives (19/2) twice independently (in *Cameraria* and *Skytanthus*). This transformation series could be interpreted as a progressive increase in the length of the connective tissue, but there is no information about the possible function of this structure, which could be involved in the pollination process.

In terms of floral complexity, there are different degrees of fusion of the carpels within the tribe. According to the MPTs, the ancestral condition for the carpel arrangement is apocarpous (31/2), which uniquely derived into secondary syncarpous (31/1) in *Allamanda* and independently also derived into partially syncarpous (31/3) in the CTC. This pattern is also present among other genera in the family, where the ancestral condition is the apocarpous that derives into syncarpous (Endress et al., 1996). One could then interpret a tendency toward fusion of the carpels within the family. In our analysis, carpel fusion (31/3) is correlated (occurs in the same internode) with lowest number of ovules per carpel (33/0). Neverthe-

less, there is a previous step in the reduction of ovule number (33/1) that is not correlated to any degree of carpel fusion.

EVOLUTION OF THE CHARACTERS RELATED TO DISPERSAL

Fallen (1985, 1986) proposed that within Plumerieae, the basic fruit type is water dispersed. The texture of the fruit (in this case of the mesocarp) and color of the exocarp, as well as the presence/absence of wings in the seed, are features that can be directly associated to dispersal. In the tribe, the mesocarp (44) can be woody, leathery, spongy, or fleshy. The interpretation of the evolution of this character in the consensus tree is ambiguous regarding the ancestral condition (woody or spongy), but in all cases fleshy and leathery fruits are always interpreted as independently derived conditions. The spongy texture, which has been associated with water dispersal (Gunn & Dennis, 1976; Ridley, 1990), could be the ancestral condition in the tribe, but it could also have appeared one to three times depending on the MPT and the optimization. At the same time, in all the MPTs, the winged seeds must be interpreted as the plesiomorphic condition, with three lost independently in *Anechites*, *Skytanthus acutus* Meyen, and *Thevetia amazonica* Ducke–*T. bicornuta* Müll. Arg. Furthermore, the compression of the seeds on both faces (54/0), which can facilitate wind dispersal, is the ancestral state. The compression of the embryo was lost in one side (54/1) in *Thevetia* and was completely lost (54/2) in *Cascabela*, suggesting that mechanisms other than anemochory are derived. In summary, our results support the hypothesis that anemochory is the ancestral mechanism of dispersion, while zoochory and hydrochory are derived within the tribe.

There are reports that *Thevetia*, with leathery mesocarp (44/1), is dispersed by animals (e.g., Fallen, 1986, reported that *T. ahouai* is consumed by monkeys), which corroborates zoochory as one of the most derived dispersal mechanisms in Plumerieae. Fleshy-colored fruits have been associated with animal dispersal (Fahn, 1982; Ridley, 1990). Although there are no reports of dispersal mechanism for species of *Cascabela*, the presence of fleshy mesocarp (44/2) with black exocarp (42/3) and the sister relationship with *Thevetia* suggest zoochory.

Colored fruits in the tribe are not restricted to leathery or fleshy mesocarps; within *Cerbera*, with spongy mesocarp (44/3), *C. manghas* L. has red exocarp (42/2), whereas *C. floribunda* K. Schum. and *C. odollam* Gaertn. have blue exocarp (42/0). The origin of a red exocarp is homoplastic (derived also in the *Thevetia* clade), which leads to the question of the chemical nature of the color. Although this genus has colored exocarp, there are reports of water dispersal (Gunn & Dennis, 1976; Leeuwenberg, 1999), which can be correlated to the spongy texture of the mesocarp. In *Cameraria*, this condition can be interpreted as a result of an independent origin or as a common origin with respect to CCC. *Cameraria* and *Cerberiopsis* also share compressed fruits (40/1), but this condition is unambiguously optimized as having two independent origins. It has been suggested that the combination of spongy and flattened fruits allows them to be dispersed by wind or water (Veillon, 1971; Fallen, 1985). The sister relationship of *Cerbera* and *Cerberiopsis* would support the idea of hydrochory in *Cerberiopsis*.

The interpretation of seed dispersal mechanisms for *Allamanda*, which also has spongy mesocarp, is complicated given the wide diversity of morphologies of fruits and seeds among its species. In *A. cathartica* L., the spongy mesocarp is very thin, whereas in the CCC, it is well developed. Nevertheless, Fallen (1986) suggested hydrochory for this genus based on the spheroid shape of the fruit. The possible independent origin of the spongy mesocarp in combination with winged seeds (50/1) suggests that the dispersal mechanism for the genus is anemochory. Although the wings in the seeds of *A. cathartica* are heavy and contradict this idea, some species of *Allamanda* have papyraceous wings, which supports the hypothesis of wind dispersal. However, other species in the genus (e.g., *A. martii* Müll. Arg., *A. oenotheraefolia* Pohl., *A. puberula* A. DC.) have an exocarp with thorny projections, which could suggest zoochory (Mauseth, 1988).

CONCLUSIONS

The phylogenetic hypothesis for the tribe can be correlated to previous subtribal classifications, and the monophyly of subtribes Plumeriinae and Thevetiinae is supported by several synapomorphies. Allamandinae is accepted as a subtribe based on the sister position of *Allamanda* with respect to Thevetiinae. Nevertheless, this subtribe is taken as provisional due to conflict with molecular data.

Both *Cascabela* and *Thevetia* are supported as monophyletic so long as *T. pinifolia* is transferred to *Cascabela*. The combination and contrast of character states that support both clades (*Thevetia* and *Cascabela*) allow us to prefer the recognition of two generic entities instead of only *Thevetia* with two subgenera. *Cascabela* has five species, all of them present in Mexico and Central America except for two species endemic to Mexico. *Thevetia* has two species native to

South America and one growing from Mexico to South America. The sister group of the *Cascabela–Thevetia* clade remains uncertain, with two equally parsimonious possibilities: *Cerbera–Cerberiopsis* or *Anechites–Cameraria*. Similarly, the phylogenetic hypothesis of *Cascabela* at species level does not have hierarchical structure in the consensus. Therefore, the incorporation of a greater amount and type of information (morphological and molecular data and more species) is suggested. There is a need for continued research regarding macromorphology and micromorphology, which will allow us to propose new characters and re-evaluate the primary homology hypotheses. In the same way, it is necessary to invest in efforts to gather field observations and in experiments to improve our knowledge of reproductive and dispersal biology.

This cladistic analysis corroborates the relevance of the use of the morphology in phylogenetic reconstruction, recovering groups postulated by traditional taxonomy, as well as by molecular or combined phylogenies.

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Appendix 1. List of species. Numbering system applies to Appendices 1 and 2 only.

1. *Carissa macrocarpa* (Eckl.) A. DC.
2. *Allamanda cathartica* L.
3. *Anechites nerium* (Aubl.) Urb.
4. *Cameraria latifolia* L.
5a. *Cascabela gaumeri* (Hemsl.) Lippold
5b. *C. ovata* (Cav.) Lippold
5c. *C. pinifolia* (Standl. & Steyerm.) Alvarado-Cárdenas & Ochot.-Booth, comb. nov.
5d. *C. thevetia* (L.) Lippold
5e. *C. thevetioides* (Kunth) Lippold
6a. *Cerbera floribunda* K. Schum.
6b. *C. odollam* Gaertn.
6c. *C. manghas* L.
7. *Cerberiopsis candelabra* Vieill. ex Pancher & Sebert
8. *Himatanthus obovatus* (Müll. Arg.) Woodson
9. *Mortoniella pittieri* Woodson
10a. *Plumeria obtusa* L.
10b. *P. rubra* L.
11a. *Skytanthus acutus* Meyen
11b. *S. hancorniaefolius* (A. DC.) Miers
12a. *Thevetia ahouai* (L.) A. DC.
12b. *T. amazonica* Ducke
12c. *T. bicornuta* Müll. Arg.

Appendix 2. Index to collections examined. Dagger (†) indicates specimens used in SEM analysis.

Acevedo, R. 249 (XAL) (2), 392 (MEXU) (5d), 953 (MEXU) (5b), 1029 (MEXU) (5b), 1298 (MEXU) (5b); Acosta 84 (MEXU) (5d); Acosta, S. 738 (MEXU) (5b), 806 (MEXU) (5b); Aguilar, C. 505 (MEXU) (12a), 806 (MEXU) (12a); Aguilar, G. 652 (MEXU) (12a), 1967 (MEXU) (12a); Aguilar, J. 55 (MEXU) (5b), 301 (MEXU) (5a); Aguilar, R. 230 (NY) (5b), 276 (MEXU) (5b), 287 (MEXU) (5b); Ahern's 21736 (NY) (6b); Alegria 79 (MEXU) (12a), Alexander 249 (NY) (5b), 2163 (MEXU) (5c); Alexiades 1113 (NY) (5d); Almeda 287 (NY) (2); Alvarado 164 (NY) (5d); Alvarado-Cárdenas s.n. (MEXU†) (6b), 19 (MEXU†) (10b), 113 (MEXU†) (5b), 901 (MEXU) (5e) 901 bis (MEXU†) (2); Alvarado-Cárdenas et al. 20 (MEXU) (5e), 38 (MEXU) (5d); Alvarez, D. 63 (MEXU) (5a), 81 (MEXU) (12a), 424 (MEXU) (12a), 1210 (NY) (5a), 1509 (MEXU) (5a), 1624 (MEXU) (5a), 1734 (MEXU) (5a), 3894 (MEXU) (12a), 9126 (MEXU) (5a); Ambrosio, 289 (XAL) (2); Ankli 321 (MEXU) (5a); Annable 3380 (NY) (6b), 2864 (NY) (5d); Antonio, T. 1259 (NY) (12a); Aquino 98AA (MEXU) (5d), 170 (MEXU) (5b); Araquistain, M. 252 (MEXU) (5d), 2954 (MEXU) (5b), 3074 (MEXU) (5d), 3393 (MEXU) (12a); Argüelles 1055 (MEXU) (5e); Aristeguieta, L. 4690 (NY) (12a); Armstrong 1 (NY) (10b); Aronso, J. 7724 (MO) (11a); Atwood, J. & Nelly 16 (MEXU) (9); Ayora 45 (MEXU) (5a).

Bacab 103 (MEXU) (5a), 121 (MEXU) (5d), 159 (MEXU) (12a); Balam 13 (MEXU) (5a), 99 MEXU) (5a), 421 (MEXU) (5a); Balanza 237 (Z) (7); Balcázar, T. 259 (MEXU) (12a); Balick, J. 1708 (NY) (12a); Balleza 7785 (MEXU) (5b), 8482 (MEXU) (5b), 8563 (MEXU) (5b); Bangham 636 (NY) (6b); Barajas 106 (MEXU) (5b); Barlow 29-10B (MEXU) (5d); Bartlett, H. 16436 (NY) (12a); Bates, D. 1685 (NY) (5d); Baumann-Bodenheim et al. 5097 (NY) (6b), 15297 (NY) (6b), 9779 (NY, Z) (7); Becerra s.n. (MEXU) (5b); Beck 1192 (NY) (6b), Beck, G. 2664 (MO) (12b); Bedel 2628 (MO) (3), Benítez 3107 (MEXU) (5b), 3137 (MEXU) (5b), 3806 (MEXU) (5b); Berlín s.n. (MEXU) (5e); Bernardi 482 (NY) (5d), 526 (NY) (5d), 20737 (NY) (5a); Bettle, A. 26128 (MO) (11a), 26129 (MO†) (11a), 26152-153 (MO) (11a); Billiet, F. & B. Jadin 5343 (MO) (11a); Black, G. & Ledoux, P. 50-10295 (MO, NY) (12b); Blanco 241 (XAL) (10b), 291 (XAL) (10b); Blanco, C. 971 (NY) (12a); Blum 983 (NY) (12a), 3987 (NY) (5d); Boege 99 (MEXU) (5e), 1912 (MEXU) (5d); Bojórquez 668 (MEXU) (5b); Bonpland 1341 (NY) (12a); Boom 6957 (NY) (5d), 10017 (NY) (5d); Boone 1205 (MEXU) (5b); Borden 24037 (NY) (6b); Boufford 25219 (NY) (6b); Brace 243-516 (NY) (5d); Breckon, J. 2045 (MEXU) (12a); Breedlove 28782 (MEXU) (12a), 34495 (NY) (12a), 51620 (MEXU) (5b); Brenes s.n. (NY) (5b), 17184 (NY) (5d), 22845 (NY) (5b); Brian 1239 (NY) (6c); Brigada Dioscóreas 2450 (MEXU) (12a); Brigada Vázquez 759 (MEXU, NY) (12a); Britton 1709 (NY) (5d); Britton 10025 (NY) (5d), 12954 (NY) (5d); Bruff 1107 (MEXU) (5b), 1278 (MEXU†); Brunner 1352 (MO) (12c); Buchholz, J. T. 1017 (NY) (6b); Bunting, G. 1277 (NY) (12a), 5033 (NY) (12a), 5110 (NY) (12a), 10215 (NY) (12a), 12767 (NY) (3), 12427 (NY) (12a), s.n. (NY) (12a); Bye, R. 15456 (MEXU) (12a).

Cabrera 24 (MEXU) (12a), 164 (MEXU, XAL) (2), 408 (MEXU) (5a), 445 (MEXU) (5a), 1098 (MEXU) (10a), 1171 (MEXU) (5a), 1262 (MEXU) (5a), 1273 (MEXU) (12a), 13505 (MEXU†) (12a), 2181 (MEXU) (5a), 2181 (NY) (5a), 2307 (MEXU) (12a), 2374 (MEXU) (10a), 2464 (NY, XAL) (4), 2485 (MEXU) (5a), 2475 (MEXU) (10a), 2581 (MEXU) (10a), 4667 (MEXU) (5a), 4789 (MEXU) (12a), 5933 (MEXU) (5b), 6327 (MEXU) (5a), 7223 (MEXU) (5a), 7381 (MEXU) (5b), 8036 (MEXU) (5d), 8919 (NY) (5d), 9041 (MEXU) (12a), 9640 (MEXU) (5a), 10250 (MEXU) (5a), 10747 (MEXU) (5a), 10747 (NY) (5a), 11328 (MEXU) (5d), 11696 (MEXU) (5a), 11793 (MEXU) (5a), 11798 (XAL) (4), 12592 (MEXU) (12a), 12661 (MEXU) (12a), 13494 (MEXU) (12a), 13505 (MEXU) (12a), 13622 (MEXU) (5d), 15442 (MEXU) (5a); Calderón Salvador 880 (NY) (5d); Callejas, R. 4915 (NY) (12a), 4936 (NY) (12a); Callejas, R. et al. 5010 (NY) (3); Calónico 3903 (MEXU) (5e), 4173 (MEXU) (5b), 5012 (MEXU) (5b), 8669 (MEXU) (5e), 15442 (MEXU) (5c), 23492 (MEXU) (12a), 23666 (MEXU) (12a), 23757 (MEXU) (12a), 23845 (MEXU) (10a), 23877

(MEXU) (5a), 24705 (MEXU) (12a); Calzada, J. 2375 (MEXU) (5d), 2666 (MEXU) (12a), 3861 (XAL) (10b), 4522 (MEXU) (5d), 5342 (XAL) (10b), 5644 (MEXU) (5d), 6326 (MEXU) (5d), 6335 (MEXU) (5d), 6422 (MEXU) (12a), 6586 (MEXU) (5a), 7819 (MEXU) (12a), 8429 (XAL) (10b), 9830 (MEXU) (12a), 10096 (XAL) (10b), 11169 (XAL) (2), 11516 (MEXU) (5d), 15016 (MEXU) (12a), 15430 (MEXU) (12a), 16071 (MEXU) (5b), 16713 (MEXU) (12a), 17690 (MEXU) (12a), 2375 (NY) (5d); Campos 133 (MEXU) (5d), 133 (MEXU) (5d), 2868 (MEXU) (5a), 2869 (MEXU) (5a); Campos-Villanueva 3722 (MEXU) (12a), 5162 (MEXU) (5b); Cancino 9 (MEXU) (5e); Cano 420 (MEXU) (5b); Carnevali, G. 5515 (MEXU) (12a); Carr 2217 (NY) (6b), s.n. (NY) (6b); Carranza 585 (MEXU) (5d); Carrasquilla 196 (MEXU) (12a); Carter 3163 (MEXU) (5d); Castañeda 41 (MEXU) (5e), 479 (MEXU) (5d); Castillo, G. s.n. (MEXU) (5d), 241 (XAL) (2), 406 (MEXU†) (12a), 2595 (MEXU) (5d), 3745 (MEXU) (12a), 5338 (XAL) (10b), 5864 (XAL) (10b), 6444 (XAL) (10b), 9311 (MEXU) (5b); Cayeros 226 (MEXU) (5e); Cedillo 21 (XAL) (2), 449 (MEXU, NY) (5b), 490 (NY) (5b), 728 (MEXU) (5b), 1006 (MEXU) (12a), 1385 (MEXU) (12a), 2872 (MEXU) (12a); Cedillo & Torres, R. 1445 (MEXU) (5e); Chan, C. 289 (XAL) (4), 536 (XAL) (2), 989 (MEXU) (5a), 1134 (XAL) (2), 1381 (MEXU) (5a), 3652 (MEXU) (12a), 3712 (MEXU) (12a); Chanek 33 (MEXU†) (5d); Charlotte 539 (NY) (5d); Chavelas ES4274 (MEXU) (12a), ES4216 (MEXU) (12a); Cházaro 1269 (MEXU) (5d), 3066 (XAL) (2), 3350 (XAL) (10b), 4836 (XAL) (10b); Chiang, F. 90 (MEXU) (5d), 536 (MEXU) (5b), 1021 (MEXU) (5b), 1021 (MEXU) (5b); Chiang, F. et al. F-1855 (MEXU) (5e); Chow 78136 (NY) (5d), 80206 (NY) (5d); Chrisophesen 656 (NY) (6b); Cibemiga 151 (NY) (5d); Clemens, J. 3384 (NY) (6b); Clemens, J. & M. Clemens s.n. (NY) (6c); College of Idaho s.n. (MEXU) (5b), M-935 (NY) (5e); Columbia College 1168 (NY) (11a); Conrad 2774 (MEXU) (12a); Conservator of Forest s.n. (NY) (10a); Constantino 7415 (NY) (6b); Contreras s.n. (MEXU) (5b); Contreras, E. 853 (MEXU) (5d), 8397 (MEXU†) (5a), 8397 (NY) (5a), 8483 (NY) (4); Conzatti 3951 (MEXU) (12a), 4631 (MEXU) (5e), 5278 (NY) (5e); Cooray 69073112R (NY) (5d); Correa, M. 10 (MEXU) (5a), 120 (MEXU) (5a), 260 (MEXU) (5a); Cortés 412 (MEXU) (5a), 473 (MEXU) (5a); Cortés, L. 310 (MEXU) (12a), 310 (MEXU) (12a), 392 (MEXU); Cowell 349 (NY) (12a); Crawford 566 (NY) (12a); Crawshaw, D. 284 (NY) (12b); Croat, T. s.n. (MEXU) (5d), 5032 (NY) (12a), 22561 (NY) (12a), 23527 (NY) (12a), 23561 (NY) (12a), 24907 (NY) (12a), 41864 (MEXU) (12a), 63241 (MEXU) (12a); Croat, T. & P. Duncan 16234 (MO) (3), 16361 (MO) (3); Cruz-Espinosa 899 (MEXU†) (10b); Curiel 192 (MEXU) (12a); Curran 3786 (NY) (6b); Curso de Ecología Vegetal p79 (MEXU) (12a).

Däniker 332 (Z) (7); D'Arcy, W. 16080 (MEXU) (12a); Darwin, S. 2347 (MEXU) (5a); Davidse, G. 9424 (NY) (12a); Degener 2451 (NY) (6b), 13718 (NY) (6b), 13768 (NY) (6b), 13978 (NY) (6b), 32077 (NY) (6b); Devia 845 (NY) (5d); Díaz, J. 5 (MEXU) (5d); Díaz, R. 132 (MEXU) (12a); Diego 7233 (MEXU) (5e), 7306 (MEXU) (5e); Dillon, M. & J. Teiller 5033 (MO†) (11a); Döbbeler 571 (MEXU) (5d); Dodson 5591-4261 (MO) (3); Dodson & A. Gentry 9792 (MO) (3); Donnell-Smith 2772 (NY) (5d); Dorado & Salinas F-2937 (MEXU) (5e); Dorantes s.n. (MEXU) (5d), 1001 (MEXU) (5d), 1264 (MEXU) (5d); Dressler 4292 (MEXU) (12a); Dreyer 337 (MEXU) (5a); Ducry 61 (MEXU) (5b); Duke 9989(1) (NY) (3), 11398 (MO) (12b), 11398 (NY) (12b), 12209(2) (MO, NY) (3), 15445 (MO) (3); Duna 107 (NY) (12a); Duna, D. 23285 (NY) (5d); Durán R. 657 (MEXU) (10a), 948 (MEXU) (10a), 1548 (MEXU) (5a), 2833 (NY) (5a), 2853 (NY) (5a), 3502 (MEXU) (5a); Durán, A. 136 (MEXU) (12a); Durán, C. 285 (MEXU) (5d); Dwyer s.n. (MEXU) (5b).

Eames 4 (NY) (6b); Edaño 503 (NY) (6b); Egler, W. & H. Irving 45946 (NY) (12b); Ekman 15329 (NY) (3); Elmer 11997 (NY) (6b), 12173 (NY) (6b), 12896 (NY) (6b), 16773 (NY) (6b); Elmer, M. s.n. (NY) (6b), 1051 (NY) (6b); Elorsa s.n. (MEXU) (5b), 255 (MEXU, NY) (5b), 304 (MEXU) (5b), 3064 (MEXU) (5b), 3064 (NY) (5b), 4718 (MEXU) (5b), 5035 (MEXU) (5b); Enríquez, E. 44 (MEXU) (5b), 57 (MEXU) (5b), 924 (MEXU) (5b); Erlanson 5084 (NY) (6b); Escalante, S. 1 (MEXU) (5a), 885 (MEXU) (5a), 893 (MEXU) (12a); Escobar s.n. (MO) (3); Espejel, I. 604 (MEXU) (5a); Espinosa 1187 (MO) (3); Ewan 23023 (NY) (5d).

Fernández 3428 (NY) (5d); Fernández, A. 1333 (NY) (10b); Fernández, C. 10776 (NY) (5d); Ferrera 166 (NY) (10a); Figueroa 1 (MEXU) (5b); Figueroa, R. 53 (MEXU) (5b); Fisher s.n. (NY) (5d); Florence, J. 3931 (NY) (6b); Flores, A. 704 (MEXU) (12a); Flores, A. J. 1586 (XAL) (10b); Flores, J. 8313 (XAL) (4), 9711 (MEXU) (12a); Flores-Franco 1077 (MEXU) (5b), 2801 (MEXU) (5b), 3542 (MEXU) (5b), 3672 (MEXU) (5b), 5243 (MEXU) (5b); Fonnegra, R. 2808 (NY) (12a); Forero, E. 481 (MO) (3), 565 (MO) (3), 1590 (NY) (12a); Fosberg, R. 9375 (NY) (5d), 25397 (NY) (5d), 50992 (NY) (6c), 56596 (NY) (6c); Foxworthy 42135 (NY) (6b); Fragoso, R. s.n. (MEXU) (5b), s.n. (MEXU) (5b); Franc, I. 545 (NY) (7); Franco 52 (MEXU) (5b); Freeland, J. 139 (MEXU) (5d); Frei FREI019 (MEXU) (5d); Frodin, D. 26615 (NY) (6b); Froes 30535 (MEXU) (12b); Frye 2586 (NY) (5e); Fryxell 3543 (NY) (5d); Fuchs 1697 (MEXU) (5d); Fuentes s.n. (MEXU) (5a), s.n. (XAL) (10b).

Gallardo, C. 609 (MEXU) (5b), 2226 (MEXU) (5a); Garber 959 (NY) (5d); García-Mendoza 646 (MEXU) (5b), 3221 (MEXU) (5d), 4097 (MEXU) (5b); García-Mendoza et al. 297 (MEXU) (5e); Gaumer 546 (NY) (5d), 22110 (MEXU) (12a); Genelle 899 (NY) (5e); Gentle 336 (NY) (5a), 4751 (NY) (5a); Gentry, A. 8071 (NY) (12a), 8198 (NY) (12a), 8484 (NY) (12a), 22757 (NY) (5d), 26296 (MO) (3); Gentry, A. & Dodson, C. 35722 (MO) (3), 41299 (MO) (3); Gentry et al. 43913 (MEXU†) (9); Gentry, H. 5028 (MEXU, NY) (5b), 5157 (MEXU, NY) (5b), 10929 (MEXU) (5b); Gereau R. E. 2204 (MEXU) (12a); Germán 479 (MEXU) (5b); Gilly 1 (NY) (5e), 51 (NY) (5d); Glassman 1819 (NY) (5b); Godínez 4 (MEXU) (5e); Gómez s.n. (MEXU) (5b); Gómez-Pompa 88 (MEXU) (12a), 107 (MEXU) (12a); Góngora 650 (MEXU) (12a); Gonzales, A. 209 (NY) (5d); González, E. 692 (MEXU) (5d); González, R. 282 (MEXU) (5e); González, S. 2403 (MEXU) (5b); González-Medrano 5068 (MEXU) (5b); Gónzalez-Medrano et al. F-613 (MEXU) (5e); Goreti 633 (MEXU) (5e); Granville 9604 (NY) (2), 10285 (NY) (2); Greenwood R1913 (NY) (12a); Gregory s.n. (MEXU) (5b), 324 (NY) (5b); Grether 1727 (MEXU) (5b); Guadarrama 885 (NY) (12a), 6568 (MEXU) (12a); Guaglianone et al. 468 (NY) (12c); Guerrero, B. 305 (XAL) (2), 1782 (XAL) (10b); Guerrero, S. 328 (XAL) (10b); Guillaumin, A. et al. 13181 (NY) (7); Guillen & Coria 1584 (MO†, NY) (12b); Guillespie, J. 2071 (NY) (6b), 2267 (NY) (6b), 3643 (NY) (6b), 3731 (NY) (6b); Guizar, E. 80 (MEXU) (5b), 2869 (MEXU) (5b); Gutiérrez, C. 1838 (MEXU) (5d), 4635 (XAL) (4), 5691 (NY) (4); Gutiérrez, L. 174 (MEXU†) (12a); Gutiérrez, R. 240 (MEXU) (5a); Guzmán 477 (XAL) (10b), 901 (XAL) (10b); Guzmán, M. 344 (MEXU) (5d), 411 (MEXU) (5b).

Hagen 1374 (NY) (12a); Halsted s.n. (NY) (5d); Hammel, B. & W. D'Arcy 5028 (MO) (3); Hansen, B. 1518 (MEXU) (5b), 7518 (NY) (5d), 7557 (NY) (5d); Harley et al. 26444 (NY†) (11b); Hatschbach 29551 (NY) (12c), 47443 (NY) (11b), 52491 (NY) (12c), 52730 (MEXU) (8); Hasskahl s.n. (NY) (6c); Hassler 7453 (MO, NY) (12c); Hayes, S. 277 (NY) (5d); Henry, A. 12747 (NY) (5d); Henty, E. 27211 (NY) (6b); Herb. Mus. Nat. Vindobon 5159 (NY) (5d); Heringer et al.

845 (NY) (12c); Hernández, A. C. 70 (NY) (5d), 3922 (MEXU) (5d), 9420 (MEXU) (5b); Hernández, A. D. s.n. (XAL) (10b); Hernández R. 19 (XAL) (2); Hernández del Olmo 618 (MEXU) (12a); Herrera, A. 128 (MEXU) (5d); Hesse, A. 1072 (NY) (6b); Heyden 3995 (NY) (5d); Hinton, G. s.n. (MEXU) (5b), 3792 (NY) (5c), 5716 (NY) (5c), 5732 (NY) (5c), 7579 (MEXU†) (5c), 7579 (NY) (5c), 7961 (NY) (5e), 9178 (NY) (5c), 10532 (NY) (5c), 11153 (NY) (5e), 12018 (MEXU) (5c), 12018 (NY) (5c); Hitchcock, A. 16864 (NY) (2); Hohenacker, R. 45 (NY) (6c); Holdridge 102 (NY) (5d); Hosokawa, T. 9670 (Z) (6a); Howard, R. 4179 (NY) (5d), 9301 (NY) (5d), 19088 (NY) (5d); Huft, M. et al. 1963 (MO) (3); Hughes 1940 (MEXU) (5b), 1940 (MEXU) (5b); Humbert H. 26511 (NY) (5d); Hunt 5450 (NY) (5d); Hunter, A. 437 (NY) (12a); Hurlimann H. 13 (NY) (6b), 24 (NY) (6b), 342 (NY†) (7), 624 (NY) (6b); Hutchison, C. 3123 (NY) (5d).

Ibarra 320 (MEXU) (5b).

Jack 8366 (NY) (12a); Jeffrey 688 (NY) (6b); Jenman 48-2 (NY) (5d); Jiménez, 4962 (NY) (3); Jiménez, Q. 11651 (NY) (5b); Jones 3101 (NY) (12a), 3425 (MEXU) (12a), 3425 (NY) (12a); Jorgenow 2065 (MO) (12c).

Kanchira 121 (NY) (6b), 468 (NY) (6b), 470 (NY) (6a), 1936 (NY) (6a), 2279 (NY) (6a); Kennedy 2834 (MO) (3); Kiesling 9702 (MEXU) (12c); Killeen 6997 (MEXU†) (12b); Killip s.n. (NY) (5d); King 1652 (NY) (5b); Klug 4006 (NY) (5d); Knapp 1906 (NY) (12a); Knapp & Mallet 3905 (MO) (3); Kocyan 991017 (MEXU†) (6c); Krapovickas & Cristobal 44169 (NY) (12c); Krug 7179 (SP) (5d); Krukoff 6297 (NY) (5d); Kuntze s.n. (NY) (5c), 16 (NY) (5d); Kuswata 6 (NY) (6b).

Ladd 264 (MEXU) (5c); Lammers et al. 7621 (MO, NY) (11a); Langlassé 269 (MEXU) (5b); Langman 3325 (MEXU) (5c); Lansing 2025 (NY) (5d); Lau 313 (NY) (6b); Laughlin 2667 (MEXU) (5b); Laugman 3325 (MEXU) (5d), 3325 (MEXU) (5d); Leavenworth 207 (NY) (5d); Lee 2107 (NY) (6b); Leeuwenberg s.n. (NY) (6c); Lei 787 (NY) (5d); Leonard 3640 (NY) (5d); Lewis 59 (MEXU) (12a); Lienser 3864 (NY) (5d); Limón s.n. (MEXU) (5b); Linares 3315 (MEXU) (5d), 5343 (MEXU) (5b); Liogier 26850 (NY) (3), 36134 (NY) (4); Lira 800 (MEXU) (10a), 916 (MEXU) (5a), 1045 (MEXU) (5a), 1051 (MEXU) (5a); Littmann E. 9042 (NY) (10b); Lomelí, J. A. 3367 (MEXU) (5c); López H. E. 266 (MEXU) (12a); López, M. 19 (NY) (5d); López, M. G. 109 (MEXU†) (12c); López, W. 1128 (MEXU) (5c), 1193 (MEXU) (5d); Lorea 5100 (MEXU) (5d); Lorence 3513 (MEXU) (12a), 5034 (MEXU) (1); Lorence et al. 3777 (MEXU) (5e); Lott 430 (MEXU) (5b), 1223 (MEXU) (5b), 2089 (MEXU) (5b); Lozada 74 (MEXU) (5b); Lundell 984 (NY) (5a), 1011 (NY) (12a), 1047 (MEXU) (12a); Lyonnet 550400017 (MEXU) (5b).

MacDougal 643s (NY) (12a); Machuca 639 (XAL) (10b); Madrid 105 (MEXU) (12a), 878 (MEXU) (5a), 1059 (MEXU) (5a); Magallanes 715 (MEXU) (5b), 4651 (XAL) (10b); Magaña 299 (MEXU) (12a), 478 (XAL) (2); Manzanero 1015 (MEXU) (5b), 1111 (MEXU) (5b); Mariz 489 (SP) (5d); Márquez 871 (MEXU) (5d); Marshall 6669 (NY) (5d); Martínez C. 21 (XAL) (2), 2129 (MEXU) (5b), 771 (MEXU) (5b), 2129 (MEXU) (5b); Martínez, E. 295 (NY) (5e), 1336 (MEXU) (5c), 1336 (NY) (5c), 2008 (MEXU) (12a), 3639 (MEXU†) (5c), 4588 (MEXU) (5b), 6324 (MEXU) (5d), 7162 (MEXU) (12a), 13599 (MEXU) (12a), 20343 (MEXU) (5b), 20928 (MEXU) (12a), 27042 (NY) (4), 27067 (MEXU) (5a), 27107 (MEXU) (5a), 27540 (MEXU) (5a), 30371 (MEXU) (5a), 35944 (MEXU) (5a); Martínez, E. et al. 2001 (MEXU) (5e), 35058 (MEXU) (12a); Mata 87-103 (MEXU) (5d); Matsuo 44 (NY) (6b); Matuda 3078 (MEXU) (12a), 4659 (MEXU) (5b), 31264 (MEXU) (5b); May 917 (MEXU) (12a), 904 (MEXU) (12a); Maya 1704 (NY) (10b); McPherson 3367 (MO†, Z) (7), 3823 (MO) (7), 3823 (MO) (7); Meagher 939 (MEXU†) (3); Medina 427 (MEXU) (5d); Medina, R. s.n. (MEXU†) (1); Mejía, S. M. T. 1615 (MEXU) (5d); Méndez, D. 76 (MEXU) (5a); Méndez, F. 296 (XAL) (4); Méndez, M. 943 (MEXU) (5a), 694 (MEXU) (5a); Méndez, T. 5894 (XAL) (10b); Mercles 3550 (MO) (12c); Merrill 3804 (NY) (5d); Meyen 4394 (MO) (11a); Mille 60 (NY) (5d); Miller 101 (NY) (5d); Miranda, A. 950 (MEXU) (5b); Miranda, F. 39 (MEXU) (5e), 424 (MEXU) (5b), 1509 (MEXU) (5b), 4229 (MEXU) (12a), 8075 (MEXU) (5a); Molina 224 (MEXU) (5b), 11008 (NY) (5b), 15633 (NY) (12a), 22437 (NY) (5b), 34933 (MEXU) (5b); Monroy de la Rosa 313 (MEXU) (5b); Montalvo 6376 (NY) (5b); Moraes et al. 1465 (NY) (12b); Morales 2929 (MEXU) (5b), 5028 (NY) (5b), 5726 (NY) (5b); Morales, M. 51 (XAL) (2); Moreno 776 (MEXU) (5b), 1237 (MEXU) (5b), 3629 (MEXU) (5b), 5476 (MEXU) (5b), 6845 (MEXU) (5d), 8551 (MEXU) (5d), 22181 (MO) (3), 24496 (MEXU†) (9), 24625 (MEXU) (12a), 24886 (MEXU) (9); Moreno P. 670 (MEXU) (10a), 2450 (MEXU) (5b), 6502 (MEXU) (5b), 8223 (MEXU) (5b), 139 (MEXU, NY) (5a); Mori 22132 (NY) (2); Moritz 1158 (NY) (12a); Morong 381 (MO) (12c), 642 (NY) (5d), 1168 (MO) (11a); Mostacedo, B. et al. 2641 (MEXU†) (8); Müller 1853 (NY) (5d); Muñoz et al. 2790 (MO) (11a); Murca 1797 (MO) (12b); Murillo 329 (NY) (10b).

Narváez 225 (MEXU) (5a); Nash 601 (NY) (3); Nee 18464 (MEXU) (5d), 26649 (XAL) (10b), 27085 (XAL) (2), 28273 (NY) (5b), 28477 (MEXU) (5d), 28477 (NY) (5d), 29341 (NY) (2), 29341 (XAL) (2), 35194 (NY) (5d), 37194 (NY) (12b), 41121 (NY) (5d); Neill 2572 (MEXU) (12a); Nervers 8303 (MEXU) (12a); Nevling 468 (MEXU) (5d); Niyomdham 1649 (NY) (6c); Noriega 22 (MEXU) (5c).

Ocaña 160 (MEXU) (12a); Ochoterena 271 (MEXU†) (5e); Oliveira 52 (SP) (5d); Orellana 289 (MEXU) (12a); Ortega, L. 117 (XAL†) (2), 431 (XAL) (2); Ortiz, G. 327 (MEXU) (5b); Ortiz, J. 331 (MEXU) (5a).

Palacios s.n. (MEXU) (5e); Palma s.n. (MEXU) (12a); Palmer, E. 27 (MEXU, NY) (5c), 339 (NY) (5d), 1069 (NY) (5b), 1533 (NY) (5b); Pancher s.n. (MO, NY) (7); Paniagua 610 (MEXU) (12a); Pascual 491 (MEXU) (5d); Peck 807 (NY) (12a); Pennell 4234 (NY) (12a), 9211 (NY) (5b); Peña-Chocarro 571 (MEXU) (5a); Peralta 378 (MEXU) (5c); Pere 2611 (NY) (5d); Pérez, A. 248 (MEXU) (5b), 861 (MEXU, NY) (5b); Peterson 2 (NY) (6c); Pfeifer 1677 (MEXU) (5b); Pipoly 4875 (MO) (3); Pittier 12099 (NY) (12a), 13071 (NY) (3); Plowman 13763 (NY) (10b); Poilane 1351 (NY) (5d); Pringle 4107 (NY) (5d), 6332 (MEXU, NY) (5b), 6739 (NY) (5b); Prinzie 157 (MEXU) (5b); Puch 373 (MEXU) (5a); Puga 17003 (MEXU) (5b); Puig 4646 (MEXU) (5d); Pulido s.n. (MEXU†) (5a); Purpus 3235 (NY) (5e).

Quero 2424 (MEXU) (5a), 2758 (MEXU) (12a); Quezada 1699 (MEXU) (5b); Quipuscoa 797 (NY) (5d).

Ramamoorthy 2692 (MEXU) (12a), 2067 (MEXU) (5a), 2448 (MEXU) (12a), 4307 (MEXU†) (5c); Ramírez s.n. (MEXU) (5c), s.n. (MEXU) (5c); Ramos 363 (MEXU) (12a); Ratter et al. 5941 (NY) (12c); Renson 232 (NY) (5d); Reyes-García 48 (MEXU) (5b), 761 (MEXU) (5b), 802 (MEXU) (5b), 1118 (MEXU) (5b), 1955 (MEXU) (5b), 2022 (MEXU) (12a), 2370 (MEXU) (12a), 2866 (MEXU) (5b), 5362 (MEXU†) (5b); Ribera 717 (MEXU) (5b); Rico-Gray 472 (XAL) (4); Rimachi 5784 (NY) (5d); Rimcahi 10217 (NY) (5d); Rincón 1011 (MEXU) (12a), 1613 (MEXU) (12a), 1672 (MEXU) (12a); Rinehart A. LR23351 (NY†) (6a); Ritter 4592 (MO) (12c); Rivera 2 (NY) (5d); Rivera, J. 717 (NY) (5b), 780 (NY) (5b); Robles 681 (XAL) (10b), 893 (MEXU) (12a); Robleto 775 (MO) (3), 986 (MO) (3), 1007 (MEXU) (5a), 1202 (MO) (3), 1329 (MO) (3); Rodríguez, D. 6 (NY) (5b); Rodríguez, G. s.n. (NY) (2); Romero 497 (XAL) (10b); Romero-Castañeda

6324 (MO†) (3), 9743 (NY) (5d), 6324 (NY) (3); Rosas 618 (MEXU) (5d); Rubio 2403 (MEXU) (5d); Ruenes, R. 73 (MEXU) (1); Rusby 361 (NY) (5d); Rzedowski 12233 (MEXU) (5e), 22307 (MEXU) (5c), 34203 (MEXU) (5b), 35724 (MEXU) (5c), 39849 (MEXU) (5e).

S.c. s.n. (NY†) (11a); Salas 2102 (MEXU) (5b); Saldias 570 (MO, NY) (12b); Saldivar 28 (MEXU) (5b); Salinas, F. 3856 (XAL) (10b), 4266 (XAL) (10b); Salinas & Solis-Sánchez F-3497 (MEXU) (5e); Salsedo 157 (NY) (6b); Sandino 655 (MEXU) (5d); Santana 2956 (MEXU) (5b); Santiago 617 (MEXU) (5b); Sauders 1032 (NY) (2), 1042 (NY) (2); Saynes 2674 (MEXU) (5b); Schmitz 748 (NY) (5e); Schubert 1688 (MEXU) (5a); Scolnik & Luti 671 (NY) (12b); Seibert 385 (NY) (12a); Seigler 13583 (NY) (5e); Shunsuke, B. 1241 (NY) (2); Silva 6237 (SP) (5d); Simá 1249 (MEXU) (5a), 1668 (MEXU) (5a); Sinaca, M. 2040 (MEXU) (12a); Sintenis 1650 (NY) (5d); Small 638 (NY) (5d), 8855 (NY) (5d); Smith 68 (NY) (6b), 1581 (NY) (6b), 1661 (MO, NY) (3), 1839 (NY) (6b), 4708 (NY) (6b), 5276 (NY) (6b), 5801 (NY) (6b), 7913 (NY) (6b), 8524 (NY) (6b), 8897 (NY) (6b), 9201 (NY) (6b); Soejarto 5997 (NY) (6b); Sohmer 9381 (MEXU) (5d); Sol 812 (MEXU) (12a); Solheim 1763 (MEXU, NY) (5d); Solís-Magallanes 3033 (MEXU) (5b); Sorensen 7074 (XAL) (4); Soto, J. 660 (MEXU) (5c), 1151 (MEXU) (5c), 2038 (MEXU) (5c), 3339 (MEXU) (5b), 4274 (MEXU) (5e), 4277 (MEXU) (5c); Sousa 8544 (MEXU) (1), 10961 (MEXU) (5a); Standley, P. 21615 (NY) (5b), 20839 (NY) (5b), 22012 (NY) (5d); Stevens 3663 (MEXU) (9), 22296 (MEXU†) (9), 24126 (MEXU) (12a); Steyermark 50708 (NY) (5b); Struwe 1216 (NY) (6b).

Tahir 699 (NY) (6c); Tapia s.n. (MEXU) (5a); Tapia & Carnevali 1386 (MEXU) (10a); Taylor 4403 (NY) (5b); Taylor, C. 10795 (MO) (11a); Teiller 878 (MO) (11a); Téllez 5610 (XAL) (4), 5621 (XAL) (4), 9959 (MEXU) (5b), 10472 (MEXU) (5b), 2175 (MEXU) (12a); Tenorio 14579 (MEXU) (12a), 19507 (MEXU) (5d); Tenorio & Alvarado-Cárdenas 20638 (MEXU) (5e); Tenorio & Kelly 21685 (MEXU) (5e); Tenorio et al. 21617 (MEXU) (5e); Tessmann, G. 3237 (NY) (5d); Thomas, W. et al. 4559 (MO, NY) (12b); Tiwari 349 (NY) (2); Toledo 291 (NY) (5c); Toro 451 (NY) (5c); Torres, L. 154 (MEXU) (5b); Torres, R. 6462 (XAL) (2), 11849 (XAL) (10b); Torres, R. & Tenorio 12797 (MEXU) (5e); Tovar R. C. 133b (XAL) (2), 216 (XAL) (2); Trott S. s.n. (NY) (5e); Tsui 212 (NY) (6b); Tún 1076 (NY) (10b).

Ucán 992 (XAL) (4), 3606 (MEXU) (5a).

Valle s.n. (MEXU) (5d); Vanpel 399 (NY) (6b); Vázquez 864 (XAL) (10b); Vega, A. 2802 (MEXU) (1); Ventura, F. 1239 (MEXU) (5e), 17876 (MEXU) (12a); Ventura, E.-López, E. 281 (XAL) (2), 1555 (XAL) (2), 1003 (XAL) (10b), 3611 (XAL) (10b), 21241 (NY) (12a); Villanueva 805 (XAL†) (4); Villareal 6755 (MEXU) (5b); Vink 12190 (Z) (6a).

Walther 340 (NY) (5d); Wang, Y. 3190 (NY) (6b); Wang, C. 34989 (NY) (6b); Waterhouse 226 (NY) (6b); Weber 1133 (NY) (6b); Wedel 2570 (MO) (3); Werdermann 136 (MO) (11a), 2597 (MO) (12c); White, D. 224 (MEXU, NY) (5a); Whitford 700 (NY) (6b); William, R. 2853 (NY) (6b); Williams 22455 (NY) (5b); Wilson 317 (NY) (5d), 1072 (NY) (11b), 2221 (NY) (11b); Woodson 1524 bis (NY) (12a); Worth, C. & J. Morrison 16162 (MO) (11a); Worthington 12398 (NY) (6c); Wright 1663 (MO) (3); Wurdak 300 (NY) (2).

Yuncker 4967 (NY) (10b), 6091 (NY) (2), 8284 (NY) (12a), 9134 (NY) (6b), 15106 (NY) (6b), 15730 (NY) (6b), 15867 (NY) (6b), 18325 (NY) (5d).

Zarucchi 3260 (NY) (5d), 4923 (NY) (5e); Zizumbo 151 (XAL) (10b), 1152 (XAL) (10b), 1153 (XAL) (10b); Zöllner 11373 (MO) (11a).

APPENDIX 3. Morphological characters and character states.

(0) Life form: 0 = vine; 1 = shrub; 2 = tree. This character was used by Endress et al. (1996), coding as the same character state trees and shrubs, considering the fact that both, in contrast to perennial herbs, have secondary growth and do not die in the winter. Here, we consider that trees and shrubs can be recognized as two distinct states; in natural conditions, trees are woody plants with a main axis that branches above ground, whereas in shrubs, it is not possible to distinguish a main axis because the plant branches from the base (Lawrence, 1958; Radford, 1986; Sousa & Zárate, 1988; Alvarado-Cárdenas & Ochoterena, pers. obs.). Given that some authors consider the difference between these two life forms merely by measuring the height of the plants, this feature was coded only from personal observations or from descriptions that allowed us to clearly define both states by means of the ramification pattern. The vines were used by Endress et al. (1996) as a different state, a coding with which we agree because vines generally have different physiological and biomechanical adaptations.

(1) Phyllotaxis: 0 = alternate; 1 = opposite; 2 = verticillate (additive). Phyllotaxis has been an important character in taxonomic and phylogenetic Apocynaceae works. Sennblad et al. (1998) regarded the verticillate condition as a variation of opposite leaves, because in the plants with verticillate leaves there are also opposite ones. Furthermore, they did not consider verticillate phyllotaxis as a character state because in their sampling, it would have represented an autopomorphic condition of *Nerium oleander* L. In contrast, Endress et al. (1996) considered verticillate leaves as a distinct character state, even though they pointed out that in the taxa with this phyllotaxis it is also possible to find opposite or alternate leaves, but they only grow just below the inflorescences. We take the position of Endress et al. (1996), coding the character states from leaves that are not subtending inflorescences, not only because we consider that the three states represent valid homology hypotheses, but also because the verticillate condition distinguishes *Allamanda* from the remaining genera, representing a potential synapomorphy for the genus. This character was here considered as additive because it represents a logical transitional series of decrease in internodal distances.

(2) Leaf blade shape: 0 = lanceolate; 1 = ovate; 2 = obovate. Liede (1994) coded for some subtribes within Asclepiadoideae two states for this character: "0 = nonlinear, 1 = linear." She considered that the variation in shape within the sampled taxa was restricted to linear vs. ovate-obovate (coded as nonlinear). Nevertheless, the recognition of just two character states could hide potential homologies among ovate and obovate leaves. Bruyns and Linder (1991) proposed the states "0 = lorate, 1 = deltoid" for some genera of Asclepiadoideae. Here we recognize a more comprehensive range of variation that applies to Plumerieae, coding the states according to the patterns suggested by Radford (1986).

(3) Presence of indumentum on vegetative parts: 0 = no; 1 = yes. This character and its states follow the proposal of Williams (2002).

(4) Secondary veins: 0 = exposed; 1 = immersed. The character and its states follow the proposal of Williams (2002).

(5) Presence of branching in the inflorescence: 0 = no; 1 = yes. This character has never before been incorporated into phylogenetic analyses. Among the sampled taxa, some species have inflorescences that never branch,

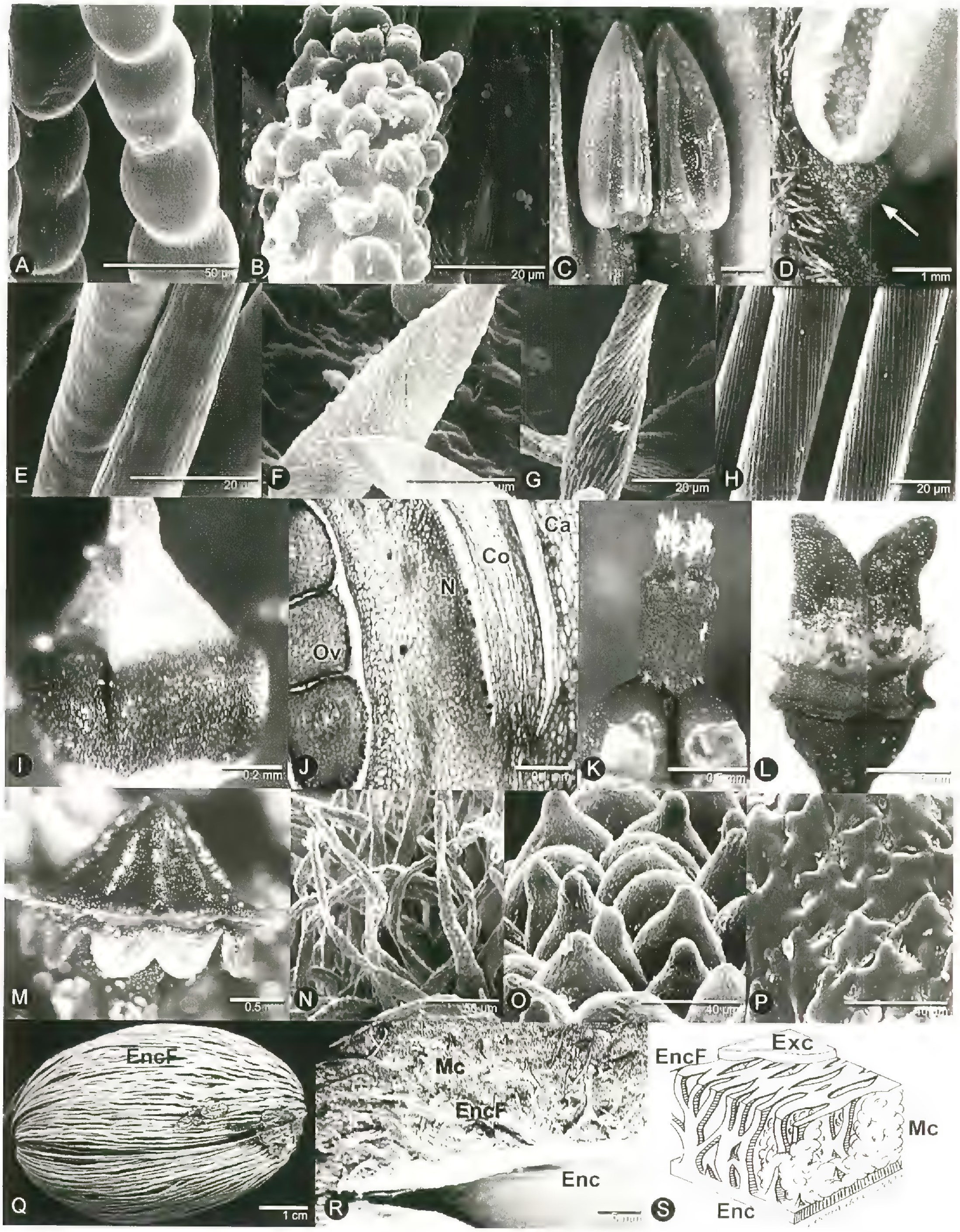

Figure 4. Selected characters and character states used in the phylogenetic analysis. A, B. Absence/presence of ornamentation of the suprastaminal trichomes (character 15). —A. Not ornamented (*Allamanda cathartica* [*Alvarado-Cárdenas 901 bis*]). —B. Ornamented (*Plumeria rubra* [*Alvarado-Cárdenas 19*]). C, D. Structures related to the anthers' support (character 17). —C. Filament (*Himatanthus oboratus* [*Mostacedo, B. et al. 2641*]). —D. Rib (arrow) (*Cerberiopsis candelabra* [*McPherson 3367*]). E–H. Absence/presence and pattern of ornamentation of the infrastaminal trichomes (characters 29 and 30). E. Absence (*Allamanda cathartica* [*Alvarado-Cárdenas 901 bis*]). —F. Verrucose (*Anechites nervum* [*Romero-Castañeda 6324*]). —G. Discontinuous striations (*Theretia amazonica* [*Guillen & Coria 1584*]). —H. Continuous striations (*Cascabela gaumeri* [*Pulido s. n.*]). I, J. Presence of nectary on the ovary (character 34, state 1). —I. General aspect of the nectary (*Cameraria latifolia* [*Meagher 939*]). —J. Longitudinal section of the flower. Ca = Calyx, Co = Corolla, N = Nectary, and Ov

whereas others produce more than one reproductive axis subtended by a single leaf.

(6) Presence of a dominant axis in inflorescences that branch: 0 = no; 1 = yes. Among the inflorescences that branch, two patterns can be distinguished. In some cases, the inflorescences develop a main axis (sympodial growth), whereas in others, the branching axes reach the same or more or less the same size and, hence, it is not possible to recognize a main axis. Species without branching inflorescences were coded as inapplicable (-).

(7) Length of the bracts subtending the flowers (mm): 0 = 0.5–4.0; 1 = 4.0–11.5; 2 = 11.5–20.0 (additive). This character has been used in taxonomic works, but not in phylogenetic analyses. Here we defined and coded the character states according to the results of descriptive statistics (see "Material and Methods"). Furthermore, the states were coded as additive because they can imply a logic transitional series of increase or decrease.

(8) Sepal form: 0 = ovate; 1 = obovate. The character states are defined according to Radford (1986). Our observations showed that sepal form is constant at species level and that both character states are unambiguously assignable.

(9) Sepal length (mm): 0 = 0.5–4.0; 1 = 4.0–11.0; 2 = 11.0–20.0 (additive). Williams (2002) coded this character for species in tribe Echiteae, recognizing the states: "0 = minute (0–3 mm), 1 = foliaceous (5–15 mm)." Within Plumerieae, we suggest an adjustment of those intervals according to descriptive statistics. At the same time, the measurements were modified starting from 0.5 mm instead of zero because to suggest a sepal length of 0 mm could be interpreted as absence of the structure, which is never the case. This character was coded as additive because there is a potential transitional series of increase or decrease.

(10) Presence of glands at the tip of the sepals: 0 no; 1 = yes. In Apocynaceae, the existence of glands at the tip of the sepals is a rare condition, occurring in only a few genera, among which are *Anechites* (Fallen, 1983) and *Plumeria* (Woodson, 1938a). This character has not been used before in cladistic analyses, but its presence in the same position and in species of the same tribe allows us to postulate it as a potential homologous condition.

(11) Presence of colleters on calyx: 0 = no; 1 = yes. Colleters are important structures generally employed in taxonomic treatments and phylogenetic studies. Endress et al. (1996) considered the variation in sepal colleter number to recognize three conditions within this character: "0 = absent, 1 = 20–50, 2 = 5." Instead, Williams (2002) considered their position and number, recognizing the following states: "0 = absent, 1 = numerous and alternate to the sepals, 2 = solitary and opposite to the sepals." Both proposals cannot be applied to species in Plumerieae because the number of colleters is very variable among individuals from the same species (e.g., from absent to 65). On the other hand, when colleters are present in members of this tribe, they are always opposite to the sepals. Therefore, in this study we only consider absence or presence.

(12) Corolla shape: 0 = hypocrateriform (Fig. 1E)**; 1 = funnelform-tubular; 2 = funnelform-campanular** (Fig. 1C, D)**.** The different shapes of the corolla follow the proposals of Font Quer (1982), Radford (1986), and Sousa and Zárate (1988). The corolla of *Thevetia amazonica* Ducke has been considered as funnelform (Gensel, 1969), but here it is coded as hypocrateriform, because the throat expands only at the staminal region, but it recovers its diameter outside this region, as it occurs in other flowers considered as hypocrateriform (e.g., *Catharanthus roseus* (L.) G. Don or *T. ahouai* (L.) A. DC.).

(13) Presence of suprastaminal appendages: 0 = no; 1 = yes. The term "suprastaminal appendages" was coined by Pichon (1948a) and used by Gensel (1969), referring to the structure that sometimes develops above the anthers. The term "corolline corolla," used by Endress et al. (1996), corresponds to this same condition. These structures have taxonomic and phylogenetic importance. Williams (2002) suggested three states: "0 = absent, 1 = reduced to a callous ridge, 2 = extended into a staminode," but only the first and third apply to our sampling. Therefore, we are coding absent/present as Endress et al. (1996).

(14) Suprastaminal appendages shape (Fig. 3): 0 = deltoid; 1 = oblanceolate; 2 = digitiform. The shape of these structures was somehow considered by Williams (2002; see previous character), but he combined in his states a mixture of qualities: shape, size, and texture. Here, we adjust the shapes according to personal observations and following Radford (1986).

(15) Presence of ornamentation on the suprastaminal trichomes: 0 = no (Fig. 4A)**; 1 = yes** (Fig. 4B)**.** The ornamentation on the suprastaminal trichomes has been reported within the tribe for species of *Himatanthus* and *Plumeria* (Pichon, 1948b), but was never before employed in cladistic analyses. Our personal observations show that these ornamentations are present in other taxa not previously reported (Table 3) and that their absence or presence is constant within species.

(16) Presence of a structure supporting the anther: 0 = no; 1 = yes. The anthers in Plumerieae can be sessile or supported by a filament or a projection of the corolla called rib (Fallen, 1986; Williams, 2002). Williams coded two characters related to the structures of support. One was the presence of a filament, considered to be one of three states: "0 = minute (0–1 mm), 1 = medium (3–6 mm), 2 = long (10 mm or more);" the other was the presence of ribs, coding them as absent/present. However, both structures (filaments and ribs) occupy the same position and apparently have the same function, and, therefore, we considered filaments and ribs as alternative homologous conditions (next character). On the other hand, a length of 0 mm could be interpreted as sessile anthers, and at the same time in our

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= Ovule. K–M. Relative position of the stigmata (characters 35, 36, 38). —K, L. Not convergent (K, *Mortoniella pittieri* [*Moreno 21496*]; L. *Skytanthus acutus* [*s. coll., s.n.*]). —M. Convergent (*Cascabela gaumeri* [*Pulido s.n.*]). N–P. Shape of the stigmata cells (character 37). —N. Filiform (*Carissa macrocarpa* [*Medina s.n.*]). —O. Conic (*Cerbera manghas* [*Alvarado-Cárdenas s.n.*]). —P. Irregularly flattened (*Plumeria rubra* [*Alvarado-Cárdenas 19*]). Q–S. Fruit layers of *Cerbera* (characters 44/3, 45/2, 46/1, 47/1). —Q. General aspect of the fruit after removing the exocarp (*Cerbera odollam* [*Rinehart A. LR23351*]). —R. Longitudinal section of the fruit after removing the exocarp showing the endocarp with the fibers extending toward the spongy mesocarp (*Cerbera odollam* [*Rinehart A. LR23351*]). —S. Schematic section of the fruit showing all layers. Enc = Endocarp, EncF = Endocarp fibers, Mc = Mesocarp, Exc = Exocarp.

sampling ribs measure more than 1 mm, so the rank for the states is here adjusted.

(17) Shape of the structure supporting the anther: 0 = filamentous (Fig. 4C); **1 = rib** (Fig. 4D). In the absence of ontogenetic or anatomical studies, we consider that the filaments and the ribs have an equivalent function and position. Thus, we postulate that both states, cylindrical and not rigid vs. flattened and rigid restricting the movement of the anthers, represent alternative homologous conditions.

(18) Anther dehiscence position: 0 = introrse (Fig. 4C); **1 = latrorse** (Fig. 4D). The character and its states are taken from Endress et al. (1996) and Williams (2002).

(19) Shape of the upper extension of the connective: 0 = obtuse; 1 = deltoid; 2 = filamentous. Williams (2002) coded the absence/presence of an apical connective without recognizing differences among them. Our personal observations show that in all the studied species of the tribe, the upper extension of the connective is always present, but it differs in form among the species. Shapes are coded according to Radford (1986).

(20) Apical connective color tone: 0 = clear; 1 = dark. This character has been used only in a taxonomic treatment (Fallen, 1983). Personal observations indicate that the presence of a dark tone in the apical connective could be the result of the oxidation of some chemical compound(s) and it is a constant condition shared by several species.

(21) Apical connective arrangement: 0 = free; 1 = connate. The fusion of the apical connective of the anthers has been mentioned for some species of *Cerbera* (Pichon, 1948a; Leeuwenberg, 1999) and *Thevetia* (Gensel, 1969; Fallen, 1986), but it has not been used in cladistic analyses. Our observations corroborate the previous works, and, therefore, we include this character for the first time in a phylogenetic context.

(22) Pollen grain diameter (μm): 0 = 20–50; 1 = 50–80; 2 = 80–120 (additive). Several authors (Pire, 1989; Roubik & Moreno, 1991; Williams, 1998, 2002) have regarded the pollen diameter as a useful character to identify and suggest relationships among genera. Van der Ham et al. (2001) considered two states in their cladistic analysis: "0 = small (3 μm), 1 = big (6 μm)," while Williams (2002) recognized three states: "0 = 20–35 μm, 1 = 40–75 μm, 2 = 75–100 μm." Here we assigned the intervals for the character states in agreement with the results of the statistical analysis (see "Material and Methods"). The character was coded as additive because it is possible to hypothesize a transitional series of increase or decrease.

(23) Tectum continuity: 0 = eutectate (perforate); 1 = subtectate (heterofoveolate to microreticulate). Endress et al. (1996) coded this character with six states. Nevertheless, the states they proposed considered a wider sampling that included more hierarchical levels. In addition, we believe that, in their coding, they included as the same attributes conditions that could be considered as different characters. For instance, they regarded as part of the tectum continuity attributes that we believe to be related to ornamentation (scabrate, undulate, verrucate, etc.). Here, we decided to recognize only the condition related to the continuity of the tectum, which in our sampling can be eutectate (imperforate or perforate) or subtectate (foveolate or reticulate). In our sampling, the ornamentation is more or less constant within the species.

(24) Exine thickness (μm): 0 = 1.0–2.0; 1 = 3.0–4.0. This character is used widely in palyno-taxonomic works, but it has not been included in cladistic analyses of the family. Even though it is variable within species, our observations show that within Plumerieae, the exine thickness has constant intervals at a specific level with a clear gap between the intervals, so it is not necessary to do a statistical analysis to recognize the two character states.

(25) Presence of endofissures in the nexine: 0 = no; 1 = yes. Endress et al. (1996) used this character to describe patterns of the inner exine, considering four states: "0 = smooth, 1 = finely granulate to verrucate, 2 = coarsely granular to verrucate, 3 = with fissures." We consider only the absence/presence of fissures (endocracks) because, although they are easily appreciable under optical and scanning electronic microscopy (displaying the same irregular pattern in *Cascabela* and *Thevetia*), at this moment we do not have observations for the inner ultrastructure that allow us to define variants in texture.

(26) Presence of a depression in the mesocolpium: 0 = no; 1 = yes. The coding of this character was taken from Endress et al. (1996).

(27) Presence of infrastaminal appendages: 0 = no; 1 = yes. The coding of this character was taken from Endress et al. (1996) and Williams (2002).

(28) Shape of infrastaminal appendages: 0 = cylindrical; 1 = quadrangular; 2 = semicircular. The character is included for the first time in a cladistic analysis, based on personal observations that show that the shape of the infrastaminal appendages is constant within species and variable amongst them, revealing its potential phylogenetic information. The definition of states follows the terminology of Radford (1986).

(29) Presence of ornamentation on the infrastaminal trichomes: 0 = no (Fig. 4E); **1 = yes** (Fig. 4F–H). The internal trichomes of the flower have not been widely studied in detail within Apocynaceae. Bruyns (2000) reported the microscopic characteristic of trichomes on the corolla of species from tribe Ceropegieae, coding the absence/presence and the ornamentation of modified trichomes. Here we adjust these attributes according to our observations. The presence/absence of ornamentation is constant within species.

(30) Infrastaminal trichome ornamentation pattern: 0 = verrucose (Fig. 4F); **1 = discontinuous striations** (Fig. 4G); **2 = continuous striations** (Fig. 4H). Within the species that display ornamentations on the infrastaminal trichomes (previous character), the pattern of the ornamentation is also constant within species and it varies amongst them. The species that lack ornamentation on the infrastaminal trichomes were coded as inapplicable (-).

(31) Carpel grouping: 0 = congenitally syncarpous; 1 = postgenitally syncarpous; 2 = apocarpous; 3 = partially syncarpous. The character and its states were modified according to our sampling from Endress et al. (1996).

(32) Ovary position: 0 = superior; 1 = semi-inferior. This character has a great taxonomic importance, and it has not been used in cladistic analyses. *Himatanthus*, *Mortoniella*, and *Plumeria* are the only genera in the tribe that share a semi-inferior ovary. Pichon (1948a, 1950a) and Leeuwenberg (1994) suggested that this character could point to a possible relationship among those genera.

(33) Number of ovules per carpel: 0 = 2; 1 = 4–6; 2 = 20–40 (additive). The number of ovules per carpel is a character that has not been used in previous phylogenetic works. It has constant intervals within species and gaps amongst them, which allow us to propose the states even without having to do descriptive statistics. This character is considered as additive, suggesting a hypothesis of logic transition to increase or reduction of ovule number.

(34) Presence of nectary: 0 = no; 1 = yes (Fig. 4I, J). The coding of this character and its states is taken from Endress et al. (1996) and Sennblad et al. (1998). Several authors (Woodson, 1938a; Standley & Williams, 1958) did not report a nectary in *Cameraria latifolia*, but our observations revealed that an external portion of the ovary wall has differences in coloration and cellular type. This tissue is here interpreted as nectary because its characteristics resemble the ones for other species where a nectary is generally reported.

(35) Stigmata shape: 0 = semispherical; 1 = cylindrical to fusiform (Fig. 4K); **2 = conic** (Fig. 4L, M). The term "stigmata" was used by Boiteau and Allorge (1978) referring to the sterile apical part of the stigmatic head. This structure has cellular differences with respect to the rest of the stigma and fulfills a particular function (Fallen, 1986). It is the first time that this character is employed in a cladistic analysis, and we recognize three characters that represent different attributes and potential homologies within the structure: shape (35), position (36), and cell shape (37). The shape of the stigmata is constant in all the species checked. The cylindrical or fusiform shapes occupy only about one third of the stigma, while the conical shape is a massive structure that constitutes half or more than half of stigma.

(36) Relative position of the stigmata: 0 = not convergent (Fig. 4K, L); **1 = convergent** (Fig. 4M). Among the species in the tribe, at least two constant patterns of stigmata arrangement can be distinguished. In not-convergent stigmatas, the lobes are parallel and independent, not touching each other. In contrast, in convergent stigmatas, the lobes are in full contact with each other, sometimes even fused at the base.

(37) Stigmata cells shape: 0 = filiform (Fig. 4N); **1 = conic** (Fig. 4O); **2 = irregularly flattened** (Fig. 4P). The shape of the stigmata cells presents different patterns amongst different species, which can unambiguously be defined as different states of the character.

(38) Secretory region shape: 0 = cylindrical to elliptic (Fig. 4K, L); **1 = lobulate** (Fig. 4M). This character was used by Boiteau and Allorge (1978) to differentiate subfamily Plumerioideae from Tabernaemontanoideae, but it has not been used in phylogenetic analyses. Here, we adopt the proposal of those authors because, within the tribe, there are clear and constant differences in the shape of the secretory region.

(39) Receptivity pattern: 0 = level 1; 1 = level 2. This character follows the proposal of Fallen (1986) and Endress et al. (1996).

(40) Fruit shape in cross-section: 0 = elliptic to circular; 1 = strongly compressed. This character was used in cladistic analyses for other families within the order Gentianales (Bremer, 1992; Ochoterena, 2000). The character is implemented here because the states are easy to recognize and not ambiguous. The compressed fruits differ from the circular or elliptical ones by a clear compression on both planes.

(41) Shape of non-compressed fruits: 0 = fusiform; 1 = linear; 2 = subspheroid (Fig. 1B); **3 = reniform** (Fig. 1F). Klackenberg (1998), when considering the fruit margin, recognized two character states: "0 = follicles with straight margin" and "1 = follicles with sinuate margin." Liede (1994) instead used the entire form of the fruit, also recognizing two states: "0 = obclavate" and "1 = fusiform." Here we adjust these proposals according to the shapes observed within Plumerieae. The linear fruits have a more or less constant diameter along them, only thinned in the apical part; the fusiform fruits are thinner at both ends. The reniform and subspheroid shapes do not have a uniform diameter along the length of the fruit; the reniform shape is wider than long and clearly displays two lobes.

(42) Exocarp color: 0 = blue; 1 = brown; 2 = red; 3 = black. The characteristic color of the fruits was employed by Williams (2002), coded as: "0 = brown," "1 = red," and "2 = black." We adjust the character states according to our sampling. We consider that the colors of the fruit represent valid homology hypotheses because they appear to be due to the presence of specific compounds.

(43) Presence of lenticels on the exocarp: 0 = no (Fig. 1F); **1 = yes** (Fig. 1B). We believe that this character has potential phylogenetic information because its presence or absence seems to be constant within species and variable among them. The lenticels on the exocarp can be seen even when the fruits are not mature.

(44) Mesocarp consistency: 0 = woody; 1 = coriaceous; 2 = fleshy; 3 = spongy (Fig. 4R, S). Endress et al. (1996) considered that the variation of fruit consistency is due to the mesocarp, an idea that we support. These authors coded the texture as: "0 = fleshy" or "1 = dry or woody." Although it is possible that some properties of the texture can be modified when the fruits dry, the characteristics that remain suggest differences in the cellular type of the mesocarp among species. We consider that it is possible to distinguish more than two characters states, from Endress et al.'s "dry or woody" condition. Potgieter and Albert (2001) considered that the fruits of *Cerbera manghas*, *C. odollam*, *Cerberiopsis candelabra*, and *Thevetia peruviana* (= *Cascabela thevetia*) have a sclerified mesocarp; our observations from *Cascabela* and *Cerbera* indicate that the sclerified consistency corresponds to the endocarp (see character 47), and the middle layers have a fleshy and spongy texture, respectively. On the other hand, *C. candelabra* does not have sclerified layers.

(45) Endocarp texture: 0 = non-sclerified; 1 = sclerified thin; 2 = sclerified thick (stony) (Fig. 4R). Endress et al. (1996) coded this character with two states: "0 = non-sclerified" and "1 = sclerified." We consider that among the genera that have fleshy mesocarp (those coded as sclerified by Endress et al.), it is possible to recognize two states: the fruits of *Cerbera* and *Cascabela* have a thicker and more rigid endocarp than those of *Thevetia*. Strictly speaking, the definition for "stony" implies the presence of stone cells (Font Quer, 1982), but given the lack of anatomical studies, we provisionally assess this state by the rigidity and the thickness of the structure, because we believe that the differences in texture among those genera could be due to a different cellular nature. The species with woody mesocarp were coded as inapplicable to avoid weighting of characters, because in that case, both layers (mesocarp and endocarp) have the same texture, a condition that we believe that could be correlated.

(46) Endocarp external surface: 0 = smooth; 1 = ornamented (Fig. 4Q–S). In tribe Plumerieae, most of the species have a smooth endocarp, but in the species of *Cerbera* and *Thevetia*, this layer is ornamented.

(47) Endocarp forming a network with the mesocarp: 0 = no; 1 = yes (Fig. 4Q–S). The presence of endocarp forming a network with the mesocarp is not common among the species of Apocynaceae. Potgieter and Albert (2001) considered that sclerified fibers of the mesocarp were responsible for the rigid consistency of *Cerbera*'s fruits, but our results show that these fibers arise from the endocarp and surround the spongy mesocarp, forming a network. The species with woody mesocarp were coded as inapplicable (see character 45).

(48) Endocarp presentation (Fig. 3)**: 0 = single; 1 = segmented.** This character has a great taxonomic importance, but it had never been used in cladistic analyses. Within Plumerieae, only the species of *Thevetia* have a segmented endocarp.

(49) Relative time of fruit dehiscence: 0 = dehiscent when mature; 1 = tardily dehiscent. Williams (2002) used this character, coding the fruits as dehiscent or indehiscent. Here we prefer to contrast relative times of dehiscence, because some fruits considered as indehiscent open after maturity, a process referred to as "delayed dehiscence" (Roth, 1977). This is the case of the drupaceous fruits of *Cascabela*, *Cerbera*, and *Thevetia* and the samaroid fruits of *Cameraria* and *Cerberiopsis*.

(50) Presence of a wing on the seed: 0 = no; 1 = yes. Endress et al. (1996) considered three states for this character: "0 = naked, 1 = winged, 2 = with cilia or hairs." Here we included the conditions present within Plumerieae.

(51) Seed wing position: 0 = concentric; 1 = at both ends; 2 = lateral opposite to the micropyle; 3 = lateral surrounding the micropyle. The position of the wing was used by Potgieter and Albert (2001), who recognized three states: "0 = not circumulate, 1 = circumulate, 2 = naked." Our observations let us recognize two variations within the not circumulate (concentric) wings, which can be established as distinct hypotheses of primary homology. In some taxa, the wing can develop opposite to the micropyle (e.g., *Plumeria*), while in others, they develop in the region of the micropyle, covering it (*Cascabela*, *Cerberiopsis*, and *Thevetia*). Taxa without seed wings were coded as inapplicable (-).

(52) Continuity of the margin of the seed wing: 0 = entire; 1 = denticulate. The characteristics of the seed wing margin were used by Pichon (1949) to diagnose the different tribes and subtribes within the family. Here we employ two of his proposals according to the states present in our sampling. Taxa without seed wings were coded as inapplicable (-).

(53) Testa texture: 0 = sclerous; 1 = coriaceous; 2 = papyraceous; 3 = crustaceous. Pichon (1949) also considered for his classification characteristics of the texture of the testa. Here we modify his proposal to include the conditions present within the tribe. The character states can be easily distinguished, and their properties seem to be maintained after drying of the specimens.

(54) Degree of embryo compression: 0 = on both sides; 1 = on one side (Fig. 3)**; 2 = apparently uncompressed** (Fig. 3) **(additive).** The compression of the embryo is implemented for the first time in a phylogenetic analysis. It refers to the shape in cross-section, which is constant within species and variable among them. The embryos with compression in both sides have a more or less linear shape; the embryos with compression have a plane face on one side and a convex face on the other; those without compression have both faces convex, looking like an ellipse. The embryo compression is not directly related to the fruit shape or the texture of the mesocarp (e.g., subspheroidal or fleshy fruits can have compressed or uncompressed embryos).

Appendix 4. Taxonomic synopsis.

Family Apocynaceae Juss., Gen. Pl. 143. 1789.
Subfamily Rauvolfioideae Kostel., Allg. Med. -Pharm. Fl. 3: 1054. 1834.
Tribe Plumerieae E. Meyer, Comm. Pl. Afr. Austr. 2: 188. 1838.
Subtribe Plumeriinae Benth. & Hook. f. Gen. Pl. 2: 685. 1876.
Subtribe Allamandinae A. DC., Prodr. 8: 318. 1844.
Subtribe Thevetiinae A. DC., Prodr. 8: 342. 1844.

Key to the *Cascabela*–*Thevetia* Complex

1a. Flowers with digitiform suprastaminal appendages; drupes pear-shaped to subspheroid, exocarp black, mesocarp fleshy, endocarp whole, thick (stony); embryo uncompressed . . . . . *Cascabela*

1b. Flowers with deltoid suprastaminal appendages; drupes reniform, exocarp red, mesocarp leathery, endocarp segmented, thin; embryo compressed on one side . . . . . *Thevetia*

1a. Cascabela Raf., Sylva Tellur. 162. 1838. *Thevetia* Adans., Fam. Pl. 2: 171. 1763, non *Thevetia* L. *Thevetia* section *Eutheretia* K. Schum., in Engler & Prantl, Nat. Pflanzenfam. 4(2): 159. 1895. *Thevetia* section *Yccotli* Kuntze, in Post & Kuntze, Lex. Gen. Phan. 558. 1903. TYPE: *Cascabela peruviana* (Pers.) Raf., Sylva Tellur. 162. 1838 [= *Cascabela thevetia* (L.) Lippold].

Trees or shrubs with milky latex. Leaves alternate, sessile or petiolate; laminae membranaceous to subleathery, with or without conspicuous secondary venation. Inflorescences terminal, corymbs compound. Sepals with colleters; corolla yellow or orange, hypogynous, funnelform, lobes sinistrorse-contorted, extended; suprastaminal appendages digitiform, villous; anthers supported by ribs, latrorse, included, agglutinated on the stigma, apical connective deltoid, dark, fused; infrastaminal appendages semicircular, hirsutulous; ovary partially syncarpic, glabrous, ovules 2 per carpel, marginal placentation, stigma conical, base 10-lobed; nectary annular. Fruit as pear-shaped to subspheroid drupes, exocarp black, with or without lenticels, mesocarp fleshy, endocarp whole, thick (stony), smooth; seeds winged, fimbrillate, testa crustaceous, embryo uncompressed.

Key to Species of *Cascabela*

1a. Leaf lamina obovate to oblanceolate.
2a. Leaves membranaceous to subleathery, glabrous, inconspicuous secondary venation; bracts glabrous; drupes not lenticellate . . . . . 1. *C. gaumeri*
2b. Leaves leathery, hirsute to tomentose, conspicuous secondary venation; bracts tomentose; drupes lenticellate . . . . . 2. *C. ovata*
1b. Leaf lamina linear to lanceolate.

3a Lamina of the leaves with inconspicuous secondary venation; corolla tube 1.0–1.8 cm.
4a. Leaves sessile, lamina linear; inflorescence peduncles 0.8–3.0 cm; bracts persistent; corolla tube internally glabrous . . . . . 3. *C. pinifolia*
4b. Leaves petiolate, lamina lanceolate to elliptic; inflorescence peduncles 0.3–0.9 cm; bracts deciduous; corolla tube internally pubescent . . . . . 1. *C. thevetia*

3b. Lamina of the leaves with conspicuous secondary venation; corolla tube 2.0–3.0 cm . . . . . 5. *C. thevetioides*

1. Cascabela gaumeri (Hemsl.) Lippold, Feddes Repert. 91: 53. 1980. Basionym: *Thevetia gaumeri* Hemsl., Icon. Pl. 1517. 1886. TYPE: Mexico. Quintana Roo: Cozumel Island, 1885, *G. F. Gaumer 7* (holotype, F not seen, F photo MEXU!). Figure 1C.

Thevetia spathulata Millsp., Publ. Field Columbian Mus., Bot. Ser. 1: 383. 1898. TYPE: Mexico. Yucatán: Yucatán, common at the port of Silam, Apr. 1895, *G. F. Gaumer 678* (lectotype, designated by Morales (2005, 43: 175), MO not seen, MO photo MO!; isotype, F).

Thevetia steerei Woodson, Amer. J. Bot. 22: 685. 1935. TYPE: Mexico. Yucatán: in low forest, Progreso, 11–15 Aug. 1932, *W. Steere 3056* (holotype, MO not seen, MO photo MO!; isotype, NY not seen, NY photo NY!).

Trees 2.0–13.0 m. Leaves petiolate; laminae oblanceolate, 10.0–12.0 × 1.9–3.0 cm, membranaceous to subleathery, glabrous, inconspicuous secondary venation. Inflorescences with 10 or more flowers; peduncles 2.0–5.0 cm, glabrous; bracts ovate, 4.0–5.7 × 3.0–4.0 mm, persistent, glabrous. Flowers with pedicels 1.6–3.5(–4.2) cm, glabrous; sepals ovate, 0.5–1.3 × 0.3–0.4 cm, glabrous; corolla greenish yellow, tube 1.5–1.7 cm, 3.0 mm diam., internally glabrous, throat 1.1–1.3 cm, 1.5 cm diam., lobes oblong, 2.6–3.0 × 2.0 cm; anthers 2.0 × 1.5 mm; ovary 1.6–1.9 × 1.7–1.9 mm, glabrous, style 1.0–1.5 cm, stigma 2.0–2.3 × 2.5 mm. Drupes 2.0–2.8 × 2.5–3.0 cm, not lenticellate; seeds 1.3–2.0 × 1.0–1.5 cm.

Distribution. Mexico (Campeche, Guerrero, Quintana Roo, Veracruz, and Yucatan) and Central America (Belize, Guatemala, and Nicaragua).

Discussion. *Cascabela gaumeri* and *C. thevetia* are morphologically very similar, but they can be easily distinguished by the shape of the laminae (oblanceolate in *C. gaumeri* vs. lanceolate to elliptic in *C. thevetia*), the hairs in the corolla tube (absent in *C. gaumeri* vs. present in *C. thevetia*), and the color of the flowers (greenish yellow in *C. gaumeri* vs. yellow or orange in *C. thevetia*).

Selected specimens examined. BELIZE. **Corozal:** Basil Jones ambergris, 15 km N de San Pedro, *S. Torre 308* (MEXU). GUATEMALA. **Petén:** Dos Lagunas, Km 7 on Ixcanrio road, *E. Contreras 8397* (MEXU, NY). MEXICO. **Campeche:** Km 6 rumbo a Nuevo Becal, *Bacab 103* (MEXU). **Guerrero:** Cerro Alquitrán, ca. del pueblo de Mazatlán, *Schwave s.n.* (MEXU). **Quintana Roo:** 0.7 km N del poblado Sabana de San Francisco, *D. Álvarez 9426* (MEXU). **Veracruz:** Cerro de Carbón, *M. Cortés 412* (MEXU). **Yucatán:** Orilla del camino Señor-San Antonio, *M. Correa 260* (MEXU).

2. Cascabela ovata (Cav.) Lippold, Feddes Repert. 91: 53. 1980. Basionym: *Cerbera ovata* Cav. Icon. 3: 35, pl. 270. 1796. *Thevetia ovata* (Cav.) A. DC., Prodr. 8: 344. 1844. TYPE: Mexico. Nueva España (holotype, MA not seen, MA photo MA!).

Cerbera alliodora Roem. & Schult., Syst. Veg. 4: 798. 1819. Syn. nov. *Cascabela alliodora* (Roem. & Schult.) Lippold, Feddes Repert. 91: 53. 1980. *Thevetia alliodora* (Roem. & Schult.) Allorge, Succulentes 21: 27. 1998. TYPE: Mexico. Guerrero: "inert Zumpango et Mescalam, in convalli Zopilote," [Apr. 1803], *F. W. H. A. Humboldt & A. J. A. Bonpland s.n.* (holotype, P not seen, P photo NY!).

Cerbera cuneifolia Kunth, Nov. Gen. Sp. 3: 224. 1818 [1819]. *Thevetia cuneifolia* (Kunth) A. DC., Prodr. 8: 344. 1844. TYPE: Mexico. Guerrero: "inert Zumpango et Mescalam, in convalli Zopilote," [Apr. 1803], *F. W. H. A. Humboldt & A. J. A. Bonpland s.n.* (holotype, P not seen, P photo MEXU!).

Thevetia cuneifolia (Kunth) A. DC. var. *andrieuxii* A. DC., Prodr. 8: 344. 1844. TYPE: Mexico. [México-Morelos]: in Mexico ad Gonacatepec, *G. Andrieux 254* (holotype, G-DC!; isotype, P not seen).

Thevetia plumeriifolia Benth., Bot. Voy. Sulphur 124, t. 43. 1845. *Cascabela plumeriifolia* (Benth.) Lippold, Feddes Repert. 91: 53. 1980. TYPE: Honduras. Gulf of Fonseca, *S. Sinclair s.n.* (lectotype, designated by Nelson (1996, 44: 60), K not seen, K photo K!).

Trees 2.0–10.0 m. Leaves petiolate; laminae obovate to oblanceolate, 5.5–17.5 × 2.5–8.5 cm, leathery, hirsute to tomentose, with conspicuous secondary venation. Inflorescences with 5 to 15 flowers; peduncles 0.5–1.3 cm, hirsute to glabrate; bracts ovate, (3.0–)5.0–7.0 × 2.0–6.0 mm, persistent, tomentose. Flowers with pedicels 2.4–7.0 cm, hirsute to glabrate; sepals ovate, 0.5–1.2 × 0.3–0.5 cm, glabrous; corolla yellow, tube 0.8–2.5 cm, 4.0 mm diam., internally glabrous, throat (0.9–)1.3–2.5 cm, 2.0–2.3 cm diam., lobes oblong, (1.2–)2.4–4.5 × (1.0–)2.2–2.5 cm; anthers 2.0 × 1.0 mm; ovary 2.0 × 4.0–5.0 mm, glabrous, style 1.5 cm, stigma 1.5–2.0 × 2.5 mm. Drupes 2.0–3.7 × 2.8–5.5 cm, lenticellate; seeds 1.8–2.0 × 1.8 cm.

Distribution. Mexico (Chiapas, Colima, Durango, Guerrero, Jalisco, México, Michoacán, Morelos, Nayarit, Oaxaca, Sinaloa, and Zacatecas), Costa Rica, El Salvador, Guatemala, Honduras, and Nicaragua.

Discussion. *Cascabela ovata* is easy to recognize by the obovate to oblanceolate leaves, with leathery

consistency, tomentose indumentum, and conspicuous secondary venation.

Selected specimens examined. COSTA RICA. **Guanacaste:** Península de Nicoya, bejuco la Islita, *Q. Jiménez 1651* (NY). EL SALVADOR. **La Libertad:** Puente Sihuatepeque, carr. Litoral de occidente, *Montalvo 6376* (NY). GUATEMALA. **El Quiché:** Río Blanco, near village of Río Blanco, *L. Williams 22455* (NY). HONDURAS. **Morazán:** Drainage of the Río Yeguare, Casa Blanca, *Glassman 1819* (NY). MEXICO. **Chiapas:** 8 km W de Tuxtla Gutiérrez, al W de la colonia Juan Crispín, *Reyes-García 1955* (MEXU). **Colima:** Manzanillo, *E. Palmer 1069* (NY). **Durango:** 6 km S de Huazamota, *S. González 2403* (MEXU). **Guerrero:** Sochopala, *Bruff 1107* (MEXU). **Jalisco:** Chimalistán, *R. Hernández 9420* (MEXU). **México:** Dolores, *Matuda 31264* (MEXU). **Michoacán:** 5–6 km NE de la desviación a Tuzantla, 14 km NE de Tiquícheo, *F. Chiang 536* (MEXU). **Morelos:** Brecha Xicatlán a Xicatlacotla, *Quezada 1699* (MEXU). **Nayarit:** Colorado de la Mora, *Benítez 3107* (MEXU). **Oaxaca:** 50 mi. S of Oaxaca on hwy., *Dwyer 14360* (MEXU). **Sinaloa:** 30 km NE de Culiacán, rumbo a la presa El Comedero, *C. Ortiz 327* (MEXU). **Zacatecas:** W de Pueblo Viejo, Cerro de Piñones ladera E, casa de J. Ayala, *Balleza 8482* (MEXU). NICARAGUA. **Boaco:** Km 101 carr. Juigalpa, el Papayal, *P. Moreno 3629* (MEXU).

3. **Cascabela pinifolia** (Standl. & Steyerm.) Alvarado-Cárdenas & Ochot.-Booth, comb. nov. Basionym: *Thevetia peruviana* (Pers.) K. Schum. var. *pinifolia* Standl. & Steyerm., Amer. Midl. Naturalist 36: 185. 1946. *Thevetia pinifolia* (Standl. & Steyerm.) J. K. Williams, Sida 17: 187. 1996. TYPE: Mexico. Michoacán: trail from Apatzingan to Tacitaro, 7 Aug. 1940, *W. C. Leavenworth 505* (holotype, F not seen, F photo MEXU!; isotypes, GH not seen, NY not seen, NY photo NY!).

Trees or shrubs 1.8–5.0 m. Leaves sessile; laminae linear, 8.0–20.0 × 0.1–0.3 cm, membranaceous, glabrous to hirsute, inconspicuous secondary venation. Inflorescences with 6 to 14 flowers; peduncles 0.8–3.0 cm, hirsute to glabrate; bracts ovate, 3.1–4.9(–6.0) × 3.0–4.5 mm, persistent, hirsutulous. Flowers with pedicels 1.0–2.7 cm, glabrous; sepals ovate, 0.5–1.1 × 3.0–4.0 mm, glabrous; corolla yellow, tube 1.0–1.8 cm, 3.0–4.0 mm diam., glabrous inside, throat 0.9–1.1 cm, 1.5–2.0 cm diam., lobes oblong, 2.4–3.7 × 2.0 cm; anthers 1.3–2.0 × 1.2 mm; ovary 2.5–3.2 × 3.0 mm, glabrous, style 1.2–1.5 cm, stigma 2.0–2.4 × 3.0 mm. Drupes 2.5–3.0 × 4.0–5.0 cm, lenticellate; seeds 1.0–1.8 × 1.0 cm.

Distribution. Endemic of Mexico in the states of Guerrero, México, Michoacán, and Puebla.

Discussion. Occasionally *Cascabela pinifolia* is incorrectly determined as *C. thevetia*, but they can be easily distinguished by the shape of the laminae (linear in *C. pinifolia* vs. lanceolate to elliptic in *C. thevetia*), the indumentum on the bracts (hirsutulous in *C. pinifolia* vs. glabrous in *C. thevetia*), and the hairs in the corolla tube (absent in *C. pinifolia* vs. present in *C. thevetia*).

Selected specimens examined. MEXICO. **Guerrero:** 29 km SW de Zirandaro, cam. Guayameo, *E. Martínez 1336* (NY). **México:** Bejucos, *G. Hinton 3792* (NY). **Michoacán:** En Las Colonias, *E. Martínez et al. 3639* (MEXU). **Puebla:** Paraje Cerro Gordo, 1 km NE de la cabecera municipal, *Castañeda 41* (MEXU).

4. **Cascabela thevetia** (L.) Lippold, Feddes Repert. 91(1–2): 52. 1980. Basionym: *Cerbera thevetia* L., Sp. Pl. 1: 209. 1753. *Cerbera peruviana* Pers., Syn. Pl. 1: 267. 1805. *Thevetia neriifolia* Juss. ex Steud., Nomencl. Bot. (ed. 2). 180. 1821. *Cascabela peruviana* (Pers.) Raf. Sylva Tellur. 162. 1838. *Thevetia linearis* Raf., Sylva Tellur. 91. 1838. *Thevetia peruviana* (Pers.) K. Schum., in Engler & Prantl, Nat. Pflanzenfam. 4: 159. 1895. *Thevetia thevetia* (L.) Millsp., Field Mus. Nat. Hist. Bot. Ser. 2: 83. 1900. TYPE: America, s. loc. et coll. (holotype, Herb. Linn. 298.1, BM not seen). Figure 1D.

Thevetia yccotli var. *glabra* A. DC., Prodr. 8: 343. 1844. Syn. nov. TYPE: Mexico. Tamaulipas: Tampico, 1827, *J. L. Berlandier 189* (holotype, G-DC!; isotype, P not seen, P image MEXU!).

Trees or shrubs 2.0–8.0 m. Leaves petiolate; laminae lanceolate to elliptical, 8.0–16.3 × 0.5–1.4 cm, membranaceous, glabrous, with inconspicuous secondary venation. Inflorescences with 6 to 8 flowers; peduncles 0.3–0.9 cm, glabrous; bracts ovate, 1.8–4.0 × 1.0–2.0 mm, deciduous, glabrous. Flowers with pedicels 2.5–3.0 cm, glabrous; sepals ovate to lanceolate, 0.5–1.3 × 0.2–0.4 cm, glabrous; corolla yellow or orange, tube 1.2–1.7 cm, 3.0–5.0 mm diam., internally pubescent, throat 0.8–1.4 cm, 1.2–1.4 cm diam., lobes oblong, 2.5–3.5 × 1.7–2.5 cm; anthers 2.0–2.5 × 1.5 mm; ovary 4.0 × 3.0 mm, glabrous, style 1.0–1.2 cm, stigma 2.0–3.0 × 2.5–3.0 mm. Drupes 2.5–3.5 × 2.1–4.5 cm, sometimes lenticellate; seeds 1.0–1.2 × 1.0 cm.

Distribution. Mexico (Campeche, Chiapas, Guerrero, Hidalgo, Michoacán, Nayarit, Oaxaca, Puebla, Querétaro, Quintana Roo, San Luis Potosí, Tabasco, Tamaulipas, Veracruz, and Yucatán), Central America (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama), South America (Bolivia, Brazil, British Guiana, Colombia, Ecuador, Perú, and Venezuela), and the Antilles (Bahamas, Cuba, Dominican Republic, Haiti, Jamaica, and Puerto Rico). Today cultivated in the tropics of the world.

Discussion. This species is frequently confused with *Cascabela thevetioides* because both have lanceolate

leaves. However, they can be distinguished by the secondary veins (inconspicuous in *C. thevetia* vs. exposed in *C. thevetioides*), the leaf indumentum (glabrous in *C. thevetia* vs. tomentose in *C. thevetioides*), and the corolla tube size (1.2–1.7 cm in *C. thevetia* vs. 2.0–3.0 cm in *C. thevetioides*). In the Linnaean herbaria there are other specimens that can be associated with *C. thevetia*: *Linn 296.4* (LINN not seen, LINN photo MEXU!) and *S-Linn. IDC 105.5* (S not seen, S photo S!). Morales (2005) considers that the holotype of this species is *Herb. Linn. 298.1* (BM), but there is no discussion supporting this decision. We are provisionally accepting his choice, mainly because we did not have access to that specimen, but we consider that it should be reviewed having all the material at hand.

Selected specimens examined. BAHAMAS: s. loc., 13 Apr 1978, *Braee 243 (516)* (NY). BELIZE. **Cayo:** El Cayo and vicinity, *Chanek 33* (MEXU). BOLIVIA. **Santa Cruz:** región de Lomerío, comunidad de San Antonio, cerca de la iglesia, *M. Toledo 291* (NY). BRAZIL. Villa Maria, *O. Kuntze s.n.* (NY). BRITISH GUIANA. s. loc., *Jenman 48-2* (NY). COLOMBIA. **Antioquia:** El Bagre, along road near airport, *Zarucchi 3260* (NY). COSTA RICA. **San José:** San Pedro, Ciudad Universitaria, *Döbbeler 571* (MEXU). CUBA. Ensenada de Mora, river valley, *N. Britton 12954* (NY). DOMINICAN REPUBLIC. Vicinity Río Arriba del Norte, N of San Juan, *R. Howard 9301* (NY). ECUADOR. In collibus prope Guayaquil, *Mille 60* (NY). EL SALVADOR. **Sonsonete:** vicinity of Nahulingo, *P. Standley 22012* (NY). GUATEMALA. **Santa Rosa:** Cenaguilla, *Heyden 3995* (NY). HAITI. Morne l'Hospital, Fortan Prince, *Holdridge 102* (NY). HONDURAS. Puerto Sierra, *P. Wilson 317* (NY). JAMAICA. **Saint Andrew:** above Mahogany Vale foot bridge, *Yuncker 18325* (NY). MEXICO. **Campeche:** 2 km W de Calakmul, camino a la Laguna, *E. Martínez et al. 27641* (MEXU). **Chiapas:** Escuintla, *Matuda 16715* (MEXU) **Guerrero:** Costa Verde, *Laugman 3325* (MEXU). **Hidalgo:** Límites de Hidalgo-San Luis Potosí, hacia Tamazuchale, *R. Hernández 3922* (MEXU). **Michoacán:** en Las Colonias, *J. Soto 4966* (MEXU). **Nayarit:** La Quita La Concordia, en el campamento Balleto, *O. Téllez 4139* (MEXU). **Oaxaca:** San Juan Atepec, 8 km de la terracería al poblado, hacia Abejones, en el Puente de Río Grande, *Alvarado-Cárdenas et al. 38* (MEXU). **Puebla:** near Coxcatlán on Cerro Ajuereado and in the adjacent valley, *Smith. C. 3640* (NY). **Querétaro:** Orilla del Río Santa María, 4 km de las mesas de Agua Fria, *A. Herrera 128* (MEXU). **Quintana Roo:** en San Miguel Cozumel, lote baldío, *Cabrera 13622* (MEXU). **San Luis Potosí:** Rascon, *Pringle 4107* (NY). **Tabasco:** Carr. W-O en el Aserradero quemado, *Calzada 2375* (NY). **Tamaulipas:** Sierra Guatemala, 1 air mile N of the square at Gómez Farias on road to Rancho del Cielo, *Sullivan 683* (NY). **Veracruz:** 2 km NE of Emiliano Zapata (Carrizal), 1 km S of hwy. Mex 140, *Hansen 7518* (NY). **Yucatán:** 1 km al SE de Tecax, sobre la carr. a Chetumal, *Cabrera 11328* (MEXU). NICARAGUA. **Chontales:** 2–3 km NE de Cuapa, *Nee 28477* (NY). PANAMA. Diablo, *Blum 3987* (NY). PERU. **Cajamarca:** ca. 35 km E of Pucara, beside Río Huancabamba, *A. Gentry 22757* (NY). PUERTO RICO. Fajardo, *Sintenis 1650* (NY). VENEZUELA. **Caracas:** Los Chaguaramos, *Ysaleny 3* (NY).

5. Cascabela thevetioides (Kunth) Lippold, Feddes Repert. 91: 53. 1980. Basionym: *Cerbera thevetioides* Kunth, Nov. Gen. Sp. 3: 223. 1818 [1819]. *Thevetia thevetioides* (Kunth) K. Schum., Nat. Pflanzenfam. 4(2): 159. 1895. TYPE: Mexico. Guerrero: Taxco, [Apr. 1803], *F. W. H. A. Humboldt & A. J. A. Bonpland s.n.* (holotype, P not seen, P photo MEXU!). Figure 1A, B.

Thevetia yccotli var. *yccotli* A. DC. Prodr. 8: 343. 1844. TYPE: Mexico. [México?]: In Mexico ad Gonacatepec, *G. Andrieux 253* (holotype, G-DC!; isotype, P not seen).

Trees 2.5–10.0 m. Leaves petiolate; laminae lanceolate, 6.0–14.0 × 0.5–1.3 cm, membranaceous, glabrous to tomentose, with conspicuous secondary venation. Inflorescences with 10 to 18 flowers; peduncles 0.6–2.1 cm, glabrous; bracts lanceolate to ovate, 0.4–1.1 × 3.0–4.0 mm, deciduous, glabrous. Flowers with pedicels 1.2–2.5 cm, glabrous; sepals ovate to lanceolate, 0.6–1.3 × ca. 0.5 cm, glabrous to hirsutulous; corolla yellow, tube 2.0–3.0 cm, 3.0–5.5 mm diam., internally glabrous, throat 1.2–1.9 cm, 1.0–1.8 cm diam.; lobes oblong-obovate, 4.0–5.3 × 3.0–3.5 cm; anthers 2.0–2.8 × 1.0–1.5 mm; ovary 2.5–4.0 × 2.0–2.5 mm, glabrous, style 1.8–2.0 cm, stigma 2.3–2.5 × 2.6–3.0 mm. Drupes 2.3–4.5 × 3.0–6.5 cm, lenticellate; seeds 1.5–1.8 × 1.5–2.0 cm.

Distribution. Endemic of Mexico in the Distrito Federal and the states of Guanajuato, Guerrero, México, Michoacán, Morelos, Oaxaca, Puebla, and Querétaro.

Discussion. This species is frequently confused with *Cascabela thevetia*, because both species have lanceolate leaves. However, they can be distinguished by the presentation of the secondary veins (exposed in *C. thevetioides* vs. inconspicuous in *C. thevetia*), the leaf pubescence (tomentose), to some extent by the flower size (corolla tube 2.0–3.0 cm in *C. thevetioides* vs. 1.2–1.7 cm in *C. thevetia*), and to some degree by the fruit size (drupes 2.3–4.5 × 3.0–6.5 cm in *C. thevetioides* vs. 2.5–3.5 × 2.1–4.5 cm in *C. thevetia*).

Selected specimens examined. MEXICO. **Distrito Federal:** Coyoacán, Jardín Botánico, *García-Mendoza 7194* (MEXU). **Guerrero:** Ajuatetlán, reserva campesina, *Godínez 4* (MEXU). **Guanajuato:** Delgado, cerca de Neutra, *Rzedowski 39849* (MEXU). **México:** Tepetitlán, *F. Ventura 1239* (MEXU). **Michoacán:** Huetamo, en las colonias, *J. Soto 4274* (MEXU). **Morelos:** lava beds near Cuernavaca, *Pringle 6332* (MEXU, NY). **Oaxaca:** Tlacolula, Tlacochahuayo, *Conzatti 4631* (MEXU). **Puebla:** Loma al NE del Calvario de Caltepec, *Tenorio & Alvarado-Cárdenas 20638* (MEXU). **Querétaro:** El Batán, *Argüelles 1055* (MEXU).

Thevetia L., Opera Var. 212. 1758, nom. cons. *Ahouai* Mill., Gard. Dict. Abr. (ed. 4). 1754. *Ahouai* Boehmer, Def. Gen. Pl. 36. 1760. *Ahouai* Adans.

Fam. Pl. 2: 171. 1763. *Thevetia* section *Ahouai* K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 159. 1895. *Plumeriopsis* Rusby & Woodson, Ann. Missouri Bot. Gard. 24: 11. 1937. *Ahovai* Pichon, Ann. Mus. Natl. Hist. Nat. 13: 227. 1948. TYPE: *Thevetia ahouai* (L.) A. DC. Prodr. 8: 345. 1844. typ. cons. [*Cerbera ahouai* L., Sp. Pl. 208. 1753].

Shrubs or trees with milky latex. Leaves alternate, petiolate; laminae subleathery, with inconspicuous secondary venation. Inflorescences terminal, corymbs simple, rarely compound. Sepals with colleters; corolla yellow to yellowish sometimes tinted with purple, hypogynous, hippocrateriform or funnelform, lobes sinistrorse-contorted, reflexed or extended; suprastaminal appendages deltoid, villous; anthers supported by ribs, latrorse, including agglutinated on the stigma, apical connective deltoid, dark, fused; infrastaminal appendages quadrangular, hirsutulous. Ovary partially syncarpic, ovules 2 per carpel, marginal placentation, stigma conical, base 10-lobed; nectary annular. Fruits as reniform drupes, exocarp red, with or without lenticels, mesocarp leathery, endocarp segmented, thin, rough; seeds with or without wing, when winged fimbriate, testa crustaceous or leathery, embryo compressed in one side.

Key to Species of *Thevetia*

1a. Leaf lamina lanceolate . . . . . 2. *T. amazonica*
1b. Leaf lamina obovate to spatulate.
  2a. Plants 1.5–13.0 m tall; leaves hirsutulous or glabrous; corolla hippocrateriform; seed winged, testa crustaceous . . . . . 1. *T. ahouai*
  2b. Plants 1.0–2.0 m tall; leaves tomentose; corolla funnelform; seeds wingless, testa leathery . . . . . 3. *T. bicornuta*

1. **Thevetia ahouai** (L.) A. DC., Prodr. 8: 344. 1844. Basionym: *Cerbera ahouai* L., Sp. Pl. ed. 2. 303. 1762. *Plumeriopsis ahouai* (L.) Rusby & Woodson, Ann. Missouri Bot. Gard. 24: 11. 1937. TYPE: Brazil. (lectotype, designated by Leeuwenberg (1993, 127: 94), Hb. Clifford: 76, Plumeria No. 1, BM not seen, BM photo BM!). Figure 1E, F.

Cerbera nitida Kunth, Nov. Gen. Sp. 3: 325. 1819. *Thevetia nitida* (Kunth) A. DC., Prodr. 8: 344. 1844. *Ahovai nitida* (Kunth) Pichon, Ann. Mus. Natl. Hist. Nat. 13: 227. 1948. TYPE: America equatorial [Colombia]: "Crescit ad ostia fluminis et juxta Turbaco Novo-Granatensium." *F. W. H. A. Humboldt & A. J. A. Bonpland s.n.* (holotype, P not seen, P photo MEXU!).

Theretia calophylla Miers, Apocyn. S. Am. 20. 1878. TYPE: Venezuela, Valle la Cura, *Mortiz 1158* (holotype, BM!).

Shrubs, rarely trees 1.5–13.0 m. Laminae obovate to elliptical, 8.0–30.0 × 3.8–8.0 cm, hirsutulous or glabrous. Inflorescences with 10 to 18 flowers; peduncles 5.0–15.0 cm, glabrous; bracts ovate, 1.8–4.5 × 2.0 mm persistent, glabrous. Flowers with pedicels 0.8–3.0 cm, glabrous; sepals ovate, 4.0–7.3 × 4.0–5.0 mm, glabrous; corolla yellow to yellowish or white, hippocrateriform, tube 2.0–3.5 cm, 3.0–4.0 mm diam., internally glabrous, lobes oblong-obovate, 0.7–1.8 × 0.6–0.8 cm, reflexed, glabrous; anthers 2.0 × 1.0 mm; ovary 2.0 × 2.0 mm, glabrous, style 1.8 cm, stigma 2.2 × 4.0–5.0 mm. Drupes 2.9–4.0 × 2.0–3.8 cm, not lenticellate; seeds 2.5 × 1.8 cm, winged, testa crustaceous.

Distribution. Mexico (Campeche, Chiapas, Guerrero, Oaxaca, Puebla, Quintana Roo, Tabasco, Veracruz, and Yucatán), Central America (Belize, Costa Rica, Guatemala, Honduras, Nicaragua, and Panama), South America (Colombia and Venezuela), and the Antilles (Cuba). Today cultivated in the tropics of the world.

Discussion. Thevetia ahouai is easily recognized by the hirsutulous to glabrous laminae, the corolla lobes reflexed, and the seeds with fimbriate wing and crustaceous testa.

Selected specimens examined. BELIZE. **Cayo:** vicinity of Cuevas S of Millionario, *T. Croat 23561* (NY). COLOMBIA. **Antioquia:** Cordoba 12 km E of Arboletes, along road to Montería, *Zarucchi 4923* (NY). COSTA RICA. **Limón:** Barranca del Colorado, N side betw. town and ocean beach, *D. Stevens et al. 24126* (MEXU). CUBA. **Santa Clara:** Soledad, Cien Fuegos, 25 *J. Jack 8366* (NY). GUATEMALA. **Izabal:** El Estor, La Mina de Exmibal, al E de El Estor, *Tenorio 14579* (MEXU). HONDURAS. **Atlantida:** vicinity of La Ceiba, *Yuncker 8284* (NY). MEXICO. **Campeche:** Calakmul, 0.9 km NW de Pioneros del Río, *Calónico 23775* (MEXU). **Chiapas:** Catzaja, Laguna de Catazaja near the hwy. betw. Villa Hermosa and Mérida and E of Bajadas Grandes, *Breedlove 28782* (MEXU). **Guerrero:** San Agustín Oapan, Solar procedente de Iguala, *Hernández del Olmo 618* (MEXU). **Oaxaca:** Cuicatlán, Cafetal El Faro, *Conzatti 3951* (MEXU). **Puebla:** Hueytamalco, El Cerro, *F. Ventura 17876* (MEXU). **Quintana Roo:** 1 km NW del entronque Mérida vía corta, *O. Téllez 2175* (MEXU). **Tabasco:** Arroyo Polo, a la derecha del puente El Coco, *Guadarrama 6568* (MEXU). **Veracruz:** 20 km E de Catemaco-Acayucán, *Gómez-Pompa 107* (MEXU). **Yucatán:** en los alrededores de la zona arqueológica de Sayil, 35 km SW de Oxkutzcab, *Cabrera 9041* (MEXU). NICARAGUA. **Zelaya:** Bluefields, 1 km W del poblado, *P. Moreno 24625* (MEXU). PANAMA. **Baru:** along ridge above Brazo Seco near Costa Rica border, *T. Croat 22561* (NY). VENEZUELA. **Trujillo:** Carr. Maracaibo–Agua Viva 16 km E de Agua Viva, *Bunting 5033* (NY).

2. **Thevetia amazonica** Duke, Arch. Jard. Bot. Rio de Janeiro 3: 247, pl. 16. 1922. TYPE: Brazil. Pará: Almeirin, *A. Duke 3058* (lectotype, designated here, MG not seen).

Shrubs 2.5–3.0 m. Laminae lanceolate, 10.0–13.0 × 1.0–1.8 cm, hirsute to tomentose. Inflorescences with 9 to 16 flowers; peduncles 0.6–1.8 cm, tomentose to glabrescent; bracts ovate, 4.9–7.0 × 3.0 4.3 mm, persistent, hirsutulous. Flowers with pedicels 2.3–

2.8 cm, glabrous; sepals ovate, 4.5–8.3 × 3.5–5.0 mm, hirsutulous; corolla yellow tinged with purple, hippocrateriform, tube 1.9–2.0 cm, 3.0–4.0 mm diam., internally glabrous, lobes oblong, 1.3–2.5 × 1.3–2.3 cm, extended; anthers 1.9–2.0 × 1.3 mm; ovary 1.3–1.7 × 1.5–1.7 mm, glabrous, style 0.7–1.0 cm, stigma 2.3 × 2.1 mm. Drupes 3.7–4.5 × 3.3–3.5 cm, lenticellate; seeds 2.4 × 1.2 cm, wingless, testa leathery.

Distribution. From Brazil to the north of Bolivia.

Discussion. *Thevetia amazonica* shares with *T. bicornuta* the tomentose indumentum and the wingless seeds with leathery testa, but *T. amazonica* is easily distinguished because it is the only species in the genus with lanceolate leaves and yellow corolla tinged with purple. In the original description, Duke listed four specimens, three of them from his own collections (*3058*, *3550*, *4918*) and the other from *Snethlage 12455*. Although we could not examine any of these specimens, we selected *Duke 3058* as the lectotype following Gensel's (1969: 49) unpublished thesis, who mentioned that it is "the most representative and complete."

Selected specimens examined. BOLIVIA. **Departamento de Beni:** San Rafael, *Beck 2664* (MO). BRAZIL. **Mato Grosso:** Mato Grosso, transpantanneire hwy., *Crawshaw 284* (NY).

3. Thevetia bicornuta Müll. Arg., Linnaea 30: 392. 1859–1860. TYPE: s. loc., *H. A. Weddell 3112* (holotype, P not seen).

Thevetia paraguayensis Britt. Ann. New York Acad. Sci. 7: 158. 1893. TYPE: [Paraguay.] Central Paraguay, *T. Morog 381* (holotype, NY!; isotypes, GH not seen, MO!).

Shrubs 1.0–2.0 m. Laminae oblanceolate to spatulate, 5.0–12.0 × 1.5–4.0 cm, tomentose. Inflorescences with 10 to 15 flowers; peduncles 2.0–3.0 cm, tomentose; bracts ovate, 4.8–9.8 × 3.1–5.0 mm, persistent, hirsutulous. Flowers with pedicels 1.5–3.0 cm, tomentose; sepals ovate, 5.0–8.5 × 4.0–5.0 mm, tomentose; corolla yellow, funnelform, tube 3.0–6.0 mm, 2.0–4.0 mm diam., internally glabrous, throat 1.5–2.0 cm, 1.0–1.5 cm diam., lobes oblong, 2.0–3.5 × 1.5–1.7 cm; anthers 2.0 × 1.5 mm; ovary 1.5 × 1.5 mm, glabrous, style 3.0–4.0 cm, stigma 2.3 × 2.0 mm. Drupes 2.0–3.0 × 2.0–2.5 cm, not lenticellate; seeds 1.5–2.0 × 1.0 cm, wingless, testa leathery.

Distribution. From eastern Brazil to Argentina, mainly in the Chaco region.

Discussion. *Thevetia bicornuta* is easily recognized by the oblanceolate to spatulate leaf laminae and the funnelform corolla.

Selected specimens examined. ARGENTINA. **Formosa:** Estan. Bouvier, Riconada, *Guaglianone et al. 468* (NY). BOLIVIA. **Santa Cruz:** Laguna Cáceres, swampy area set back from the channel of a stream or small river about 1 km W of the Río Sicuri, *Ritter 4592* (MO). BRAZIL. **Mato Grosso do Sul:** Río Paraguai, porto Manga, *Hatschbach 29551* (NY). PARAGUAY. **Alto Paraguay:** frente a Valle Mi, Riacho Mosquito, *Kiesling 9702* (MEXU).

PHYLOGENY OF APOCYNOIDEAE AND THE APSA CLADE (APOCYNACEAE S.L.)[1]

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ABSTRACT

Phylogenetic relationships were reconstructed among 59 of 77 genera of subfamily Apocynoideae and exemplars of Periplocoideae, Secamonoideae, and Asclepiadoideae (collectively the APSA clade) using sequences from four regions of the chloroplast genome (*trnL* intron and *trnL-trnF* spacer, *rpl16* intron, *rps16* intron, *matK* and 3′ half of *trnK* intron) and 16 morphological characters. Apocynoideae are resolved as paraphyletic. The five tribes recognized within this subfamily in the classification of Endress and Bruyns are all paraphyletic or polyphyletic. Seven major clades of Apocynoideae are identified. The first three include genera classified predominantly in tribes Wrightieae and Malouetieae sensu Endress and Bruyns and form a paraphyletic grade to a crown clade. The crown clade includes four clades of Apocynoideae genera classified in tribes Apocyneae, Mesechiteae, and Echiteae together with Periplocoideae, Secamonoideae, and Asclepiadoideae; the latter three constitute the traditional Asclepiadaceae. Asclepiadaceae are resolved as polyphyletic, although the node that precludes a paraphyletic Asclepiadaceae does not have bootstrap support. The clade of Secamonoideae–Asclepiadoideae is well supported as sister to a clade of three African Apocynoideae genera (*Baissea* A. DC., *Motandra* A. DC., and *Oncinotis* Benth.). There is a strong correlation between geographic distribution and phylogeny among crown clade Apocynoideae. A New World clade is composed of American genera plus the predominantly Australasian *Parsonsia* R. Br. and *Artia* Guillaumin. An Asian clade is composed of Asian, Malesian, and Australasian genera plus the north temperate *Apocynum* L. *Trachelospermum* Lem. is polyphyletic with American and Asian species nested in the New World and Asian clades, respectively. The implications of this phylogeny for the evolution of pollen aggregation and mass transfer, the traits that were used to separate Asclepiadaceae from Apocynaceae s. str., are discussed.

Key words: Apocynaceae, Apocynoideae, APSA clade, Asclepiadaceae, biogeography, evolution, molecular phylogenetics, morphology, Periplocoideae, systematics.

APSA CLADE

The APSA clade (Table 1), subfamilies Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae of Apocynaceae s.l. (Endress & Bruyns, 2000), has been resolved as a monophyletic group in all phylogenetic analyses of the family (Judd et al., 1994; Sennblad & Bremer, 1996, 2002; Civeyrel et al., 1998; Potgieter & Albert, 2001).

Robert Brown (1810) divided the genera of the APSA clade between two families, Apocynaceae s. str. (diagnosed by pollen in monads) and Asclepiadaceae (diagnosed by pollen in tetrads or pollinia; Endress, 2004). His classification was widely followed until recently (Endlicher, 1838; Schumann, 1895; Cronquist, 1981; Rosatti, 1989; Heywood, 1993).

Although never formally named under the *International Code of Botanical Nomenclature* (McNeil et al., 2006; Sennblad & Bremer [2002] coined the informal name Apocynoidina), the APSA clade was one of the first infra-familial groups recognized in Apocynaceae s.l. (Table 1). Jussieu divided Apoc-

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Table 1. Taxonomic circumscriptions. Apocynaceae s.l. includes Apocynaceae s. str. and Asclepiadaceae. Apocynaceae s. str. corresponds to subfamilies Rauvolfioideae and Apocynoideae of Apocynaceae s.l. Asclepiadaceae corresponds to subfamilies Periplocoideae, Secamonoideae, and Asclepiadoideae. The APSA clade includes the latter four subfamilies. The subfamilial classification follows Endress and Bruyns (2000).

Apocynaceae s.l.	Apocynaceae s. str.	Rauvolfioideae	
		Apocynoideae	APSA clade
	Asclepiadaceae	Periplocoideae	
		Secamonoideae	
		Asclepiadoideae	

ynaceae s.l. into three unnamed groups; one of these (diagnosed by an ovary of two free carpels, follicular fruit, and comose seeds) corresponds in circumscription to the APSA clade (Jussieu, 1789; Endress, 2004). Subsequent workers have added dextrorsely contorted corollas and lignified, basally sterile anthers (Mueller-Argoviensis, 1860), attachment of the anthers and style-head to form a gynostegium (Bentham, 1876), porate pollen (Nilsson, 1986; Nilsson et al., 1993), and the presence of steroidal alkaloids and cardenolides and absence of indole alkaloids (Heg-nauer, 1988; Endress et al., 1990) to the list of diagnostic characters, making the APSA clade one of the most easily recognized groups in Apocynaceae s.l.

Evolution of Complex Pollination Mechanisms

Prior to the application of modern phylogenetic methods, it was already widely believed that Apocynaceae s. str. are non-monophyletic with respect to Asclepiadaceae (Table 1; Schumann, 1895; Demeter, 1922; Macfarlane, 1933). Morphological comparisons of the complex and highly modified flowers of Asclepiadaceae to the simpler flowers of Apocynaceae s. str. yielded three competing hypotheses about how the former evolved from the latter. The most widely accepted hypothesis proposes that floral morphologies of Apocynoideae, Periplocoideae, and Secamonoideae represent a succession of transitional stages between the least modified flowers of Rauvolfioideae and the most modified flowers of Asclepiadoideae (Demeter, 1922; Safwat, 1962; Cronquist, 1981; Rosatti, 1989; Endress, 1994, 2001, 2004; Endress & Bruyns, 2000; Wyatt et al., 2000). In this view, the formation of the gynostegium in Apocynoideae was followed by the evolution of increasingly efficient mechanisms of pollen aggregation and mass transfer. *Apocynum* L. (with pollen borne in tetrads and simple translators) is often regarded as the first stage in this transition series (Demeter, 1922; Safwat, 1962; Nilsson et al., 1993), although Nilsson et al. (1993) hypothesized that tetrads in *Apocynum* and Periplocoideae are convergent, citing differences in pollen anatomy. (See discussion of translators of *Apocynum* in Appendix 2, char. 7.) Periplocoideae (with pollen in tetrads and spoon-shaped translators) have been interpreted as the next stage in the series. The tetrads are deposited onto the distal part of the spoon, which is attached to the pollinator by a sticky basal pad. Some genera of Periplocoideae have tetrads coherent into four pollinia, representing a further degree of pollen aggregation (Nilsson et al., 1993; Verhoeven & Venter, 1998). Secamonoideae have four pollinia attached to a clip-like translator (Civeyrel, 1994). They have been considered as transitional between Periplocoideae and Asclepiadoideae on the basis of several plesiomorphic features, including the presence of four sporangia per anther, predominantly non-linear microspore tetrads (Safwat, 1962), and the absence of a pollinial wall (a sporopollenin wall enclosing each pollinium; Verhoeven & Venter, 2001; Verhoeven et al., 2003). Asclepiadoideae are characterized by anthers with two rather than four sporangia and, thus, two pollinia attached to each translator of two arms (caudicles) connected to the clip-like corpusculum, linear microspore tetrads, and a pollinial wall (Frye, 1901; Safwat, 1962; Dan Dicko-Zafimahova, 1980; Verhoeven & Venter, 2001; Verhoeven et al., 2003).

An alternative view holds that Asclepiadaceae are polyphyletic with Periplocoideae nested in Rauvolfioideae and Secamonoideae–Asclepiadoideae nested in Apocynoideae (Schumann, 1895; Wanntorp, 1988). In this scenario, similarities shared by the pollination mechanisms of Periplocoideae and Secamonoideae–Asclepiadoideae, such as the presence of translators and aggregate pollen, are convergent. This hypothesis is based on a single character, the presence of unlignified, fully fertile anthers (similar to those of many Rauvolfioideae) in Periplocoideae, and the frequent misinterpretation of flowers of Periplocoideae as lacking a gynostegium (Huber, 1983; Swarupanandan et al., 1996). In fact, Periplocoideae have gynostegia (Nilsson et al., 1993; Endress & Bruyns, 2000) and all the other diagnostic characters of the APSA clade except lignified anthers.

Macfarlane (1933) also proposed a polyphyletic Asclepiadaceae but suggested that Periplocoideae and Secamonoideae–Asclepiadoideae are each most closely related to different genera of Apocynoideae, *Isonema* R. Br. and *Alafia* Thouars and *Baissea* A. DC. and *Oncinotis* Benth., respectively. The characters he cited to support these relationships, however, were either misinterpreted by him or are

widespread in Apocynoideae and therefore uninformative for determining relationships.

All phylogenetic studies firmly reject the idea that Periplocoideae are nested in Rauvolfioideae and instead support their inclusion in the APSA clade (Judd et al., 1994; Sennblad & Bremer, 1996, 2002; Civeyrel et al., 1998; Potgieter & Albert, 2001). However, only those studies with the sparsest samples of Apocynoideae have resolved a monophyletic Asclepiadaceae (Judd et al., 1994; Civeyrel et al., 1998; Lahaye et al., 2005). Studies with larger samples of Apocynoideae either do not resolve the position of Periplocoideae in the APSA clade (Potgieter & Albert, 2001) or place them as sister to various genera of Apocynoideae (Sennblad & Bremer, 1996, 2002; Sennblad et al., 1998). *Baissea* (Apocynoideae) is resolved (but with weak support) either as sister to Secamonoideae–Asclepiadoideae (Potgieter & Albert, 2001), sister to Secamonoideae (Sennblad et al., 1998, a study that did not sample Asclepiadoideae), or intercalated between Secamonoideae and Asclepiadoideae (Sennblad & Bremer, 2002).

Apocynoideae

Bentham (1876) was the first to name a group that corresponds to the current circumscription of Apocynoideae as tribe Echitideae, one of three tribes of Apocynaceae s. str. Schumann (1895) first treated it as subfamily Echitoideae, a precedent that has been followed by all subsequent workers except Woodson (1930), who restricted Apocynoideae to *Apocynum* and closely related genera (distinguished by pollen in tetrads), placing all the monad-bearing genera in Echitoideae.

The inclusion of three genera, *Holarrhena* R. Br., *Spirolobium* Baill., and *Carruthersia* Seem., in Apocynoideae has been controversial. Schumann (1895) placed *Holarrhena* in Plumerioideae (Rauvolfioideae) because it lacks lignified anthers. Pichon (1950b) concurred and further removed *Spirolobium* and *Carruthersia* to Rauvolfioideae subtribe Holarrheninae because he interpreted all three genera as lacking gynostegia, although he suggested that they represent the group of Rauvolfioideae most closely related to Apocynoideae. Endress et al. (1990) showed that these three genera do have gynostegia and that they share all the other diagnostic characters of the APSA clade (except *Holarrhena* lacks lignified anthers). Leeuwenberg (1994) and Endress and Bruyns (2000) included them in Apocynoideae.

Subdivision of Apocynoideae

The three most widely used tribal classifications of Apocynoideae (Pichon, 1950a; Leeuwenberg, 1991; Endress & Bruyns, 2000) are very different in their circumscriptions (Fig. 1). Of the less well-known recent systems, that of Lý (1986) is highly incongruent with each of these three, while that of Sennblad and Bremer (2002) is largely in agreement with the interpretations of Endress and Bruyns (2000).

Pichon (1948b) based his classification on a structure that is particularly difficult to observe, the region of the stamen by which it attaches to the style-head to form the gynostegium, which he termed the retinacle (Table 2). Although this classification is based on a single character, Pichon (1950a: 4) considered it phylogenetic ("the tribes composed of allied genera and probably monophyletic"; translated from the French) because, in his estimation, the state of the retinacle correlates well with the states of other characters.

Leeuwenberg (1994) criticized Pichon's tribal classification as artificial and asserted that it separated closely related genera on the basis of one difficult-to-observe character. He recognized three tribes, Wrighteae, Echiteae, and Apocyneae, dividing the genera of Mesechiteae sensu Pichon among the latter two (Fig. 1).

Endress and Bruyns (2000), like Pichon, emphasized characters of the retinacle in their classification (Table 2), although their observations were much more precise because they were based on scanning electron microscope (SEM) and serial sections (Fallen, 1986; Endress et al., 1990; Nilsson et al., 1993; Sennblad et al., 1998). They also incorporated the shape of the style-head (Table 2) and the results of several phylogenetic studies (Sennblad et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002). Endress and Bruyns (2000) reinstated Mesechiteae; although of the nine genera included in their circumscription, only six (three as synonyms) are among the 13 genera that constitute Mesechiteae sensu Pichon (Fig. 1). They also segregated genera from Wrightieae and Echiteae, as defined by both Leeuwenberg and Pichon, into a fifth tribe, Malouetieae (Fig. 1B).

Phylogenetic studies indicate that Apocynoideae are paraphyletic, with the genera *Wrightia* R. Br. and/or *Stephanostema* K. Schum. sister to the rest of the APSA clade (Sennblad & Bremer, 1996, 2002; Sennblad et al., 1998; Potgieter & Albert, 2001). *Holarrhena* (the only one of the three disputed genera of Pichon's subtribe Holarrheninae [Pichon, 1950b; Endress et al., 1990] included in molecular analyses to date) has been strongly supported as a member of the APSA clade (Sennblad et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002), corroborating its inclusion in Apocynoideae on the basis of morphological characters. In all these studies the

Table 2. Diagnostic retinacle and style-head shape characters used by Pichon (1948b, 1950a) and Endress and Bruyns (2000) as amended by Simões et al. (2004) to delimit tribes of Apocynoideae (names used by Pichon in parentheses). Pichon's descriptions are translated from the French.

	Pichon (1948b, 1950a)	Endress & Bruyns (2000)/Simões et al. (2004)	
	Retinacle	Retinacle	Style-head
Wrightieae (Nerieae)	brush of uniformly long trichomes	circular patch of trichomes	basal collar present
Malouetieae		circular patch of trichomes	basal collar absent
Echiteae (Parsonsieae)	visor of trichomes	horseshoe-shaped rim of trichomes	basal collar present
Mesechiteae (Ichnocarpeae)	glabrous facet	cellular fusion	5-ribbed
Apocyneae (Ecdysanthereae)	glabrous longitudinal ridge often accompanied by a visor of trichomes below	horseshoe-shaped rim of trichomes with a narrow longitudinal strip above	basal collar absent

relationships of most of the included genera of Apocynoideae were unresolved or only weakly supported. More recently, Simões et al. (2004) showed that Mesechiteae and Apocyneae sensu Endress and Bruyns (2000) are polyphyletic. They also showed that *Mandevilla* Lindl. is paraphyletic (Simões et al., 2006, 2007a), pointing to the need for reassessment of other generic circumscriptions.

Objectives

Much progress has been made in our understanding of the phylogeny of Apocynaceae s.l. since the analysis of Judd et al. (1994), but many questions about the APSA clade have remained unanswered. Relationships between Apocynoideae, Periplocoideae, and Secamonoideae–Asclepiadoideae are mostly unclear, precluding a test of the hypothesized transformation series leading to the highly modified flowers of Asclepiadoideae. The delimitation of genera and supergeneric groups within Apocynoideae has continued to be problematic, and most proposed circumscriptions have yet to be tested using molecular data. Because all results to date indicate that Apocynoideae are paraphyletic with respect to the other three subfamilies of the APSA clade, these two sets of problems (circumscriptions within Apocynoideae and relationships among the subfamilies) are inexorably linked. To address them, we have reconstructed phylogenetic relationships in the APSA clade using a greatly expanded sample of Apocynoideae and exemplars of the other three subfamilies.

Materials and Methods

Taxon Sampling

We sampled 119 species representative of the taxonomic, morphological, and geographic diversity of Apocynoideae. This sample comprises representatives from 59 of 77 currently recognized genera of Apocynoideae, including all seven genera of tribe Wrightieae, nine of 12 Malouetieae, 20 of 27 Apocyneae, four of six Mesechiteae, 18 of 23 Echiteae, and one of two genera incertae sedis (Appendix 1). Unless otherwise noted, the tribal and generic classification of Apocynoideae followed here is that of Endress and Bruyns (2000) as amended by Simões et al. (2004, 2006, 2007b, this issue) and Morales and Williams (2004). We sampled seven species of Periplocoideae, two with and five without pollinia, including taxa indigenous to Asia, Australia, and Africa, and representing five of the clades present in the phylogenetic analysis of the subfamily in this volume (Ionta & Judd, 2007, this issue). No well-corroborated tribal classifications are available for Secamonoideae that sample the subfamily adequately across its geographic range. Most previously published phylogenies have focused on taxa from Madagascar (Civeyrel, 1994; Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Lahaye et al., 2007, this issue). For this study, we chose two species of Secamonoideae, one widespread from Asia to Australia with dextrorse aestivation and without leaf colleters and the other from Asia with sinistrorse aestivation and leaf colleters. The division of Asclepiadoideae into four tribes has been well corroborated by phylogenetic analyses (e.g., Verhoeven et al., 2003); we included one species each of Fockeae and Ceropegieae, and two each of Marsdenieae and Asclepiadeae. As outgroups, we sampled 17 species from 11 genera from the tribes Carisseae, Plumerieae, and Alyxieae of Rauvolfioideae, which have been shown to be closely related to the APSA clade (Potgieter & Albert, 2001; Simões et al., 2007b). The root was placed arbitrarily at *Condylocarpon* Desf. Multiple accessions of 11 species were sequenced for

Table 3. New amplification and sequencing primers designed for this study. Primers are numbered according to their position in our alignments and labeled F for forward and R for reverse. Polymorphic sites are indicated by standard International Union of Pure and Applied Chemistry (IUPAC) codes.

cpDNA region	Primer name	Sequence
matK gene/3′ *trnK* intron	matK 681R	5′-GMBRAAAAYRGATTCGTATTCAC-3′
	matK 810F	5′-TTBATGMATTATGTKAGGTTATC-3′
	matK 1360r	5′-GTTTTHGCACAMGAAAGBCGAAG-3′
rpl16 intron	rpl16 Fseq	5′-GCTATGCTTAGTGTGTGACTCGTTGGT-3′
	rpl16 458F	5′-GAGCTTCGRGYCAAYAAAGACTRAGAAG-3′
	rpl16 467F	5′-GYCAAYAAAGACTRAGAAGATTGACTC-3′
	rpl16 588R	5′-RCATTCAYAGYTTHCATCGTTCCC-3′
	rpl16 643R	5′-TTYCGCCATCCCGAYCRGTG-3′
	rpl16 Rseq	5′-CATTCTTCCTCTATGTTGTTTACGG-3′
rps16 intron	rps16 455F	5′-GGYATGTTGCTRCCATTTWGAAMGG-3′
	rps16 520R	5′-CATTGGGTTTAGRCATTMCTTCGG-3′

one or more of the sampled loci to confirm that the cladograms of chloroplast haplotypes represent species phylogeny (Shaw & Small, 2005). Voucher specimens and GenBank accession numbers are listed in Appendix 1. This is study accession number S1774 in TreeBASE.

DNA EXTRACTION

DNA was extracted with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions (except grinding was done with sand rather than liquid nitrogen) from approximately 20–30 mg of dried leaf material, either preserved in silica gel or removed from a herbarium specimen, and eluted in 160–200 µL of buffer. DNA extracts that yielded weak or no polymerase chain reaction (PCR) products were further purified and concentrated with the QIAquick PCR Purification Kit (Qiagen) using 100–200 µL of DNA extract and eluting in 30–50 µL of buffer.

PRIMERS, AMPLIFICATION, CLONING, AND SEQUENCING

We sequenced four regions of the chloroplast genome (*matK* and the 3′ half of the *trnK* intron, *rpl16* intron, *rps16* intron, and *trnL* intron and *trnL-trnF* spacer) using published (*matK* 8F, 503F, 503R, 681F, 900F, 1309F, 1628R, and *trnk* 2R [Endress et al., 1996]; *rpl16* F71 and *rpl16* R1516 [Baum et al., 1998]; *rps*F and *rps*R2 [Oxelman et al., 1997]; *trnL* c, d, e, and *trnF* f [Taberlet et al., 1991]) and newly designed primers (Table 3).

PCR was performed in a volume of 10–25 µL containing 10 mM Tris-HCl (pH 8.6), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 4 µg/mL BSA, 0.25 µM of each primer, 2.5 U/100 µL Taq polymerase (USB), and 0.5–1.5 µL of unquantified DNA extract, diluted as necessary. For DNA extracts that yielded no or weak PCR products with this reaction mix, concentrations were increased to 1.6 mM dNTPs, 8 µg/mL BSA, 2 µM of each primer, and 5 U/100 µL Taq. The cycling program consisted of 4 minutes at 94 C followed by 35 cycles of 1 minute at 94 C, 1 minute at 55°C, 1 or 2 (*matK*/3′ *trnK*) minutes at 72 C, and ended with 7 minutes at 72°C, run on an MJ Research PTC-200 thermal cycler (Waltham, Massachusetts, U.S.A.).

We used primers rps16 F and trnK 2R in a long PCR reaction to amplify selectively the chloroplast-encoded copy of *matK*/3′ *trnK* from accessions that yielded heterogeneous sequences when amplified with primers *matK* 8F and *trnK* 2R (J. McNeal, pers. comm.). Five U/100 µL of FideliTaq (USB) proofreading polymerase was included in the high-concentration reaction mix described above, but with 2.75 mM $MgCl_2$, and cycled with the following program: 2 minutes at 94°C, 9 cycles of 10 seconds at 94°C, 30 seconds at 55°C, 6 minutes at 68°C, 15 cycles of 20 seconds at 94°C, 30 seconds at 55°C, 6 minutes, 20 seconds/cycle at 68 C, 7 minutes at 68 C (McNeal, 2005).

Unincorporated dNTPs and primers were removed either with the QIAquick PCR Purification Kit (Qiagen) or with ExoSap-IT (USB), and the purified PCR product used as the template for direct sequencing with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (v3.1; Applied Biosystems, Foster City, California, U.S.A.). Sequencing reactions were precipitated with isopropanol, resuspended in water, and analyzed on an ABI 3100 capillary sequencer (Applied Biosystems) in the Department of Organismal and Evolutionary Biology at Harvard University.

PCR products that could not be sequenced directly because of heterogeneity in lengths of simple

sequence repeats were inserted into a pGEM-T plasmid (Promega, Madison, Wisconsin, U.S.A.) and transformed into XL1-Blue competent cells (Stratagene, La Jolla, California, U.S.A.). Positive colonies were identified using blue/white screening and PCR with insert primers. Plasmids isolated with the FastPlasmid Mini Kit (Eppendorf, Hamburg, Germany) were sequenced as described above for PCR products.

SEQUENCE EDITING, ALIGNMENT, AND ANALYSIS

Sequencing traces were edited and contigs assembled with Sequencher v. 4.2 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A). Alignments were constructed manually using Se-Al v. 2.011a (Rambaut, 1996) and indels coded using the simple indel coding method (Simmons & Ochoterena, 2000) as implemented in GapCoder (Young & Healy, 2003). Winclada (Nixon, 2000) was used to view and manage matrices, launch tree searches in NONA (Goloboff, 1996), map characters, and view and output trees. Unalignable regions and simple sequence repeats were excluded from analysis (Table 4). Individual sequences were treated as terminals for the separate analysis of each locus. Conspecific sequences were concatenated into species terminals for the combined four-locus analysis and the combined chloroplast and morphology analysis. We implemented two searches for most parsimonious trees (MPTs) for each matrix: (1) 1000 random addition sequences, with subtree pruning and re-grafting (SPR) followed by tree bisection-reconnection (TBR) swapping, holding a maximum of 10 equally parsimonious trees per search; and (2) 10 sequential ratchet searches (Nixon, 1999), each consisting of 200 iterations, randomly reweighting 10% of the characters and randomly constraining 10% of the nodes at each iteration. Trees from the two searches were pooled and TBR-swapped until 20,000 equally parsimonious trees were saved or until the search swapped to completion. Tree length, consistency index (CI), retention index (RI), and bootstrap support (BS) were calculated on the basis of informative characters only. Bootstrap support (Felsenstein, 1985) was calculated based on 1000 pseudoreplicate matrices, each analyzed with a single random addition sequence and TBR swapping until a maximum of 20 trees were saved (Freudenstein et al., 2004). Bootstrap frequencies were calculated based on the strict consensus trees obtained from each bootstrap replicate (Davis et al., 2004). Four individual molecular data sets were analyzed (*trnL* intron/*trnL-F* spacer, *rps16* intron, *rpl16* intron, and *matK*/3′ *trnK* intron). Congruence among these data sets was assessed by identifying contradictory nodes with greater than 50% BS support as applied by Hufford et al. (2003).

MORPHOLOGICAL MATRIX

We reconstructed the evolution of morphological characters that are relevant to two different parts of the tree topology obtained from the molecular data set (Fig. 1, Appendices 2, 3). The first group of characters distinguishes the 18 genera of the first three branches (the *Wrightia*, *Nerium* L., and *Malouetia* A. DC. clades) from the rest of the APSA clade (the crown clade; Fig. 1). The second group of characters includes those that are the basis for the hypothesis that Periplocoideae are transitional between Apocynoideae and Secamonoideae, including proposed synapomorphies of Periplocoideae and Secamonoideae–Asclepiadoideae, as well as characters that support the monophyly of the African clade and Secamonoideae–Asclepiadoideae (Fig. 1). Whenever there are alternative interpretations of character homology, we have encoded the interpretation that favors a sister relationship between Periplocoideae and Secamonoideae–Asclepiadoideae to test if the morphological characters can overturn the resolution favored by the sequence characters (Fig. 1). All characters were constructed using reductive coding, dividing composite characters into logically and/or biologically independent ones (Wilkinson, 1995; Simmons & Freudenstein, 2002), treated as non-additive, and included in a simultaneous analysis with the combined four locus molecular data set (Nixon & Carpenter, 1996). Characters were mapped with accelerated, delayed, and unambiguous optimizations on multiple MPTs. Characters were coded based on examination of specimens held at three herbaria (A, NY, and MO), spirit-preserved material under a dissecting scope and with SEM, and serial sections by the third author, and based on literature denoted with an asterisk (*) in the Literature Cited.

RESULTS

SEQUENCES

A complete or partial sequence of each of the *trnL* intron/*trnL-F* spacer, *rps16* intron, *rpl16* intron, and *matK*/3′ *trnK* intron was obtained for each studied species, except no *matK*/3′ *trnK* intron sequences were obtained for three outgroup species (*Thevetia peruviana* K. Schum., *Chilocarpus rostratus* Markgr., *Pteralyxia kauaiensis* Caum) and one ingroup species (*Ichnocarpus rhombifolius* (Markgr.) D. J. Middleton), and no *rpl16* intron sequences were included for the

Table 4. Summary of analyses. For analyses of the individual loci, conspecific haplotypes are treated as separate terminals; for the combined analyses, all conspecific haplotypes are fused into species terminals. Columns that correspond to regions that are considered unalignable are included in the count of columns in the alignment. Unknown cells are quantified as percentages of the total number of cells in the matrix, i.e., the number of terminals multiplied by the number of columns in the alignment; regions with indels are not included in this count. Columns of an alignment are excluded from the analysis because they contain homopolymers or otherwise unalignable regions. Indels are coded with the simple indel coding method of Simmons and Ochoterena (2000). For each analysis, a maximum of 20,000 MPTs was saved; analyses that yielded 20,000 MPTs are presumed to have more. The count of MPTs includes ambiguously supported topologies. Length, CI, and RI are calculated based on informative characters only.

Analysis	No. of terminals	No. of columns in alignment	% cells unknown	No. of (%) alignment columns excluded from analysis	No. of informative substitutions	No. of informative indels	No. of informative morphological characters	No. of MPTs	Length of MPTs (steps)	CI of MPTs	RI of MPTs
matK gene/3′ *trnK* intron	154	2069	2.9	34 (1.6)	629	44	0	216	2136	47	78
rpl16 intron	156	1938	1.9	796 (41)	302	50	0	> 20,000	1126	45	77
rps16 intron	163	1140	5.3	50 (4.4)	264	76	0	> 20,000	1048	48	76
trnL intron/*trnL-F* spacer	165	1295	0.085	22 (1.7)	244	107	0	> 20,000	942	49	78
Combined molecular	150	6442	4.5	902 (14)	1414	269	0	144	5189	47	76
Combined molecular plus morphology	150	6442	4.5	902 (14)	1414	269	16	144	5250	46	76

ingroup species *Allomarkgrafia brenesiana* Woodson, *Forsteronia acouci* (Aubl.) A. DC., *F. velloziana* (A. DC.) Woodson, *Odontadenia lutea* (Vell.) Markgr., and *Rhabdadenia macrostoma* (Benth.) Müll. Arg. GenBank accession numbers are given in Appendix 1. The percentage of unknown cells in each alignment ranges from 0.085% for *trnL* intron/*trnL-F* spacer to 2.9% for *matK*/3′ *trnK* intron (Table 4). A total of 4.5% of aligned cells are unknown in the concatenated matrix of all four loci (Table 4).

Heterogeneous sequences in PCR products of the *matK*/3′ *trnK* intron amplified with primers *matK* 8F and *trnK* 2R were detected in *Gymnanthera oblonga* (Burm. f.) P. S. Green and *Toxocarpus villosus* (Blume) Decne. by the presence of double peaks in the sequencing traces. The same double peaks were present in sequences from at least two independent PCR reactions from two independent DNA preparations for both species. Sequences of these two species from PCR products amplified with primers *rps16* F and *trnK* 2R lacked double peaks. These were interpreted as the chloroplast-encoded copies and included in the data set.

No conspecific haplotypes (i.e., sequences from multiple accessions of a species) were resolved as polyphyletic in analyses of any of the four individual loci (results not shown). When all conspecific haplotypes were fused into species terminals for the simultaneous analysis of all four loci, 33 substitution and indel characters that are informative when individual alleles are terminals of the analysis become autapomorphic or polymorphic within species and are thus rendered uninformative, resulting in a total of 1683 informative characters for the simultaneous analysis of all four sequence partitions (Table 4).

ANALYSES

Statistical summaries of each of the six data matrices (*trnL* intron/*trnL-F* spacer, *rps16* intron, *rpl16* intron, *matK*/3′ *trnK* intron, combined molecular, and combined molecular + morphology), are presented in Table 4. Alignment of three of the four molecular matrices was straightforward, with only a small percentage of positions (1.6%–4.4%) excluded because of ambiguity due to presence of homopolymers or unalignable regions (Table 4). The exception is the *rpl16* matrix, from which 41% of positions had to be excluded from analysis (Table 4). Six segments dispersed throughout the alignment were excluded, three homopolymers and three unalignable. The largest unalignable segment constituted of 626 positions of primarily imperfect repeats near the 3′ end of the intron.

There is a single instance of contradictory clades receiving greater than 50% bootstrap support in some of the four sequence partitions and in the combined molecular analysis. In the *rps16* and *rpl16* matrices *Heterostemma* Wight & Arn. was resolved as sister to *Asclepias* L.–*Vincetoxicum* Wolfe (77% BS *rps16*, 58% BS *rpl16*), whereas in the combined molecular analysis and in the other two partitions *Heterostemma* is resolved as sister to *Marsdenia* R. Br.–*Telosma* Coville (72% BS combined, 87% BS *trnL* intron and *trnL-F* spacer, 68% BS *matK*). All other clades in each of the four sequence partitions that contradict clades in the simultaneous analysis (Fig. 1) received less than 50% bootstrap support (data not shown).

Figure 1A presents one of the 144 MPTs from the analysis of the four locus molecular data set. Addition of the sixteen morphological characters to the molecular matrix yielded the same 144 MPTs (Table 4). Eleven of the nodes in this MPT collapse in the strict consensus tree (Fig. 1, branches marked with -/-). The bootstrap values for clades in the combined morphological and molecular analysis are usually within a few percentage points of values for the molecular analysis only (Fig. 1). Unless otherwise noted, we hereafter refer to the topology and bootstrap values of the strict consensus tree for the combined molecular and morphological analysis.

PHYLOGENETIC RELATIONSHIPS, GEOGRAPHIC DISTRIBUTIONS, AND CHARACTER EVOLUTION

The APSA clade is resolved as monophyletic with 100% bootstrap support (Fig. 1A). Of its four constituent subfamilies, three, Periplocoideae, Secamonoideae, and Asclepiadoideae, are monophyletic, as is the grouping of Secamonoideae–Asclepiadoideae (all 99%–100% BS; Fig. 1B). Apocynoideae is paraphyletic. The clade comprising the three African genera, *Baissea*, *Oncinotis*, and *Motandra* A. DC. (all Apocyneae, Apocynoideae), informally designated the African clade, is sister to Secamonoideae–Asclepiadoideae (93% BS). A crown clade including Periplocoideae, Secamonoideae, Asclepiadoideae, and all genera classified in Apocynoideae tribes Apocyneae, Echiteae, and Mesechiteae (except *Neobracea* Britton) is resolved with high support (97% BS). The crown clade is resolved with moderate support (81% BS) as sister to the *Malouetia* clade (70% BS). A sister relationship (100% BS) is found between the *Nerium* clade (unsupported by the bootstrap) and the crown–*Malouetia* clades. The *Wrightia* clade (100% BS) is sister to the rest of the APSA clade (100% BS).

Along with the Periplocoideae and the African clade–Secamonoideae–Asclepiadoideae, the crown

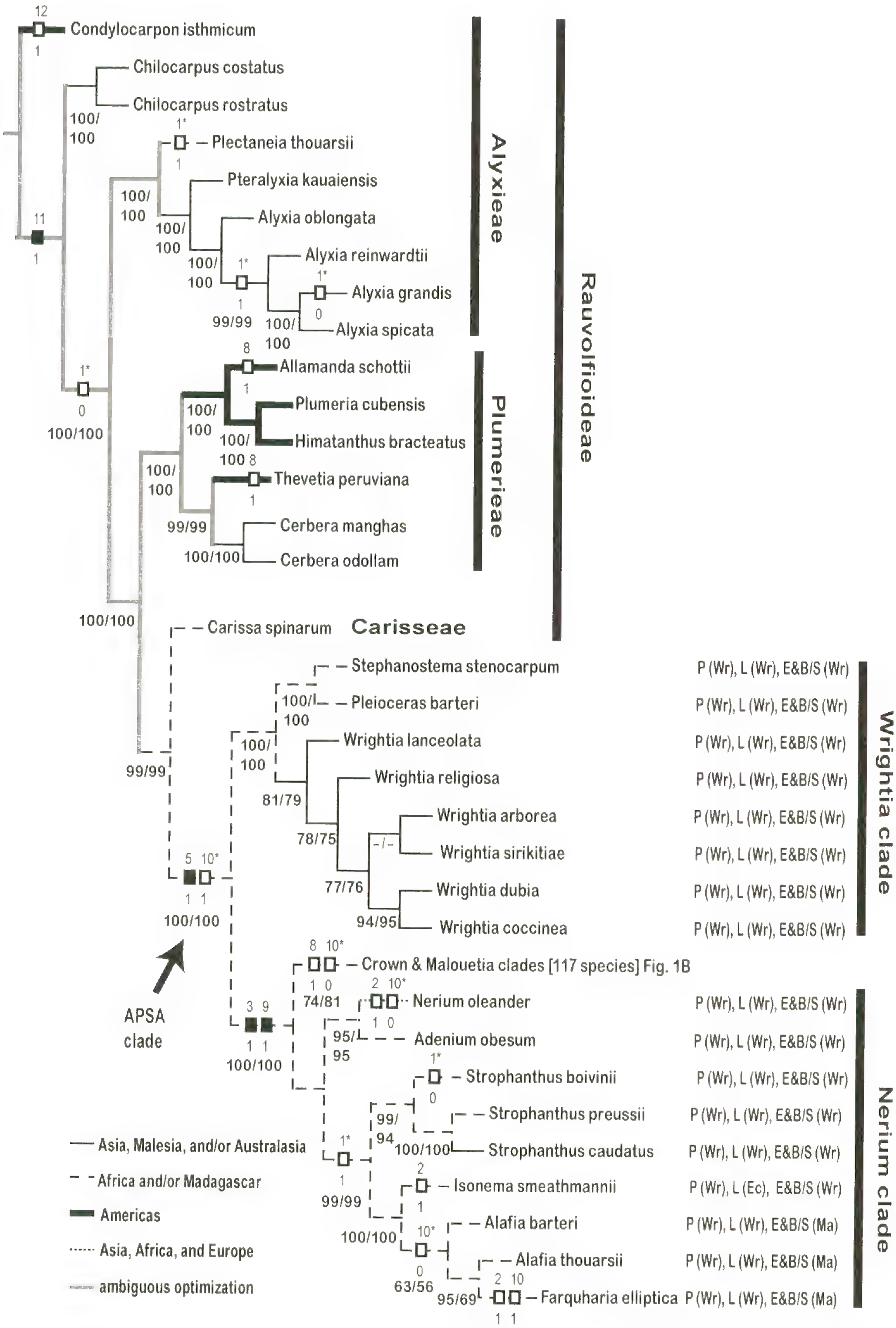

Figure 1A. One of 144 most parsimonious trees (MPTs) (L = 5189 steps, CI = 47, RI = 76) from the combined analysis of *trnL* intron/*trnL-trnF* spacer, *matK*/3′ *trnK* intron, *rps16* intron, and *rpl16* intron. The combined analysis of the four molecular markers plus 16 morphological characters yielded the same 144 MPTs (L = 5250 steps, CI = 46, RI = 76). Bootstrap values ≥ 50% are shown below the branches (molecular only/molecular + morphology). Nodes not resolved in the strict consensus of 144 MPTs are indicated with dashes instead of bootstrap values below the branches (-/-). Geographic

clade includes three additional subclades: a well-supported clade of Apocynoideae referred to hereafter as the Asian clade (99% BS), the genus *Rhabdadenia* Müll. Arg. (Echiteae, Apocynoideae; 100% BS), and a weakly supported clade of Apocynoideae designated the New World clade (58% BS; Fig. 1B). Relationships among these five primary subclades of the crown clade (African clade–Secamonoideae–Asclepiadoideae, Periplocoideae, *Rhabdadenia*, New World clade, and Asian clade) are not resolved in the strict consensus tree or not supported by the bootstrap (Fig. 1B).

Figure 1A–D shows the 16 morphological characters (Appendices 2, 3) mapped onto one of the MPTs with accelerated (ACCTRAN) optimization (fit: length of morphological matrix on MPTs from combined morphological and molecular matrices (L) = 61 steps, CI = 29, RI = 85). Geographic occurrence is mapped onto the same MPT as a non-additive character, and ancestral areas are reconstructed with unambiguous optimization (Fig. 1A–D).

Discussion

Data and Analysis

This study presents the best sampled, resolved, and supported phylogeny of the APSA clade to date (Fig. 1A–D). We have sampled 59 of 77 genera of Apocynoideae recognized by Endress and Bruyns (2000) as amended by Simões et al. (2004, 2006, 2007a) and Morales and Williams (2004), almost three times as many as included by Potgieter and Albert (2001) and Sennblad and Bremer (2002), who examined 22 and 21 genera of Apocynoideae, respectively. Our character matrix is more than 4.5 times larger, with 1683 informative molecular characters (Table 4), compared to 358 assembled by Potgieter and Albert (2001) and 237 by Sennblad and Bremer (2002).

The morphological matrix analyzed here is a special purpose one, composed of characters that have been used to separate taxa of the *Wrightia*, *Nerium*, and *Malouetia* clades from the crown clade, along with features that have been hypothesized as representing a transformation series among Periplocoideae, Secamonoideae, and Asclepiadoideae. Not surprisingly, support for the *Malouetia* clade (66%–70% BS) and for the sister group relationship of the *Malouetia* and crown clades (74%–81% BS) increases when the morphological matrix is added to the combined molecular matrix (Fig. 1B), and support for the monophyly of the African clade–Secamonoideae–Asclepiadoideae decreases from 98% BS to 93% BS (Fig. 1B). The tree topology is identical with or without the morphological characters (Fig. 1); however, we consider inclusion of morphological characters in a simultaneous analysis a stronger test of our homology assessments than excluding them from the analysis and mapping them on to a molecular tree, because the latter procedure does not consider the phylogenetic evidence of the morphological characters at all and maximizes their homoplasy (Luckow & Bruneau, 1997).

The major clades resolved in this study (see Fig. 1) are discussed below with regard to their morphology and how their constituent genera were placed in the classification systems of Pichon (1950a), Leeuwenberg (1994), and Endress and Bruyns (2000; see Table 2). Throughout this discussion, Pichon's tribes (Pichon, 1950a) will be referred to by their correct names according to the rules of priority to facilitate comparison with the other classifications.

APSA Clade (Fig. 1A)

The APSA clade is monophyletic with 100% BS support. Attachment of the anthers and style-head to form a gynostegium, lignified anthers, porate pollen, and comose seeds have all been previously identified as synapomorphies of this clade (Judd et al., 1994; Sennblad & Bremer, 1996; Potgieter & Albert, 2001). Of the characters included in our analysis (Appendix 2), the presence of lignified anthers (char. 5, 1), secondarily lost in *Holarrhena* and Periplocoideae, and the presence of a chalazal coma (char. 10, 1) are optimized as synapomorphies for the APSA clade, although the latter is ambiguous and it is equally parsimonious to optimize this feature as evolving independently in the *Wrightia* clade and in each of

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distributions are indicated as in the figure legend and mapped with unambiguous optimization. Morphological character changes are indicated by boxes on the branches with the character numbers above and character states below (see Appendix 2). The accelerated transformation (ACCTRAN) optimization is shown. White boxes indicate homoplastic changes, and black boxes indicate non-homoplastic changes. Ambiguously optimized character changes are indicated by an asterisk (*) next to the character number. Optimizations that are artifacts of coding inapplicable characters as unknown (Maddison, 1993) are indicated with a dagger (†) next to the character number. The position of each species of Apocynoideae in three recent classifications is indicated after the species name using the following code: P = (Pichon, 1950a), L. = (Leeuwenberg, 1994), E&B/S = (Endress & Bruyns, 2000), amended by (Simões et al., 2004), (Ap) = Apocyneae, (Ec) = Echiteae, (inc. sed.) = incertae sedis, (Ma) = Malouetieae, (Me) = Mesechiteae, (Ra) = subfamily Rauvolfioideae, (Wr) = Wrighteae.

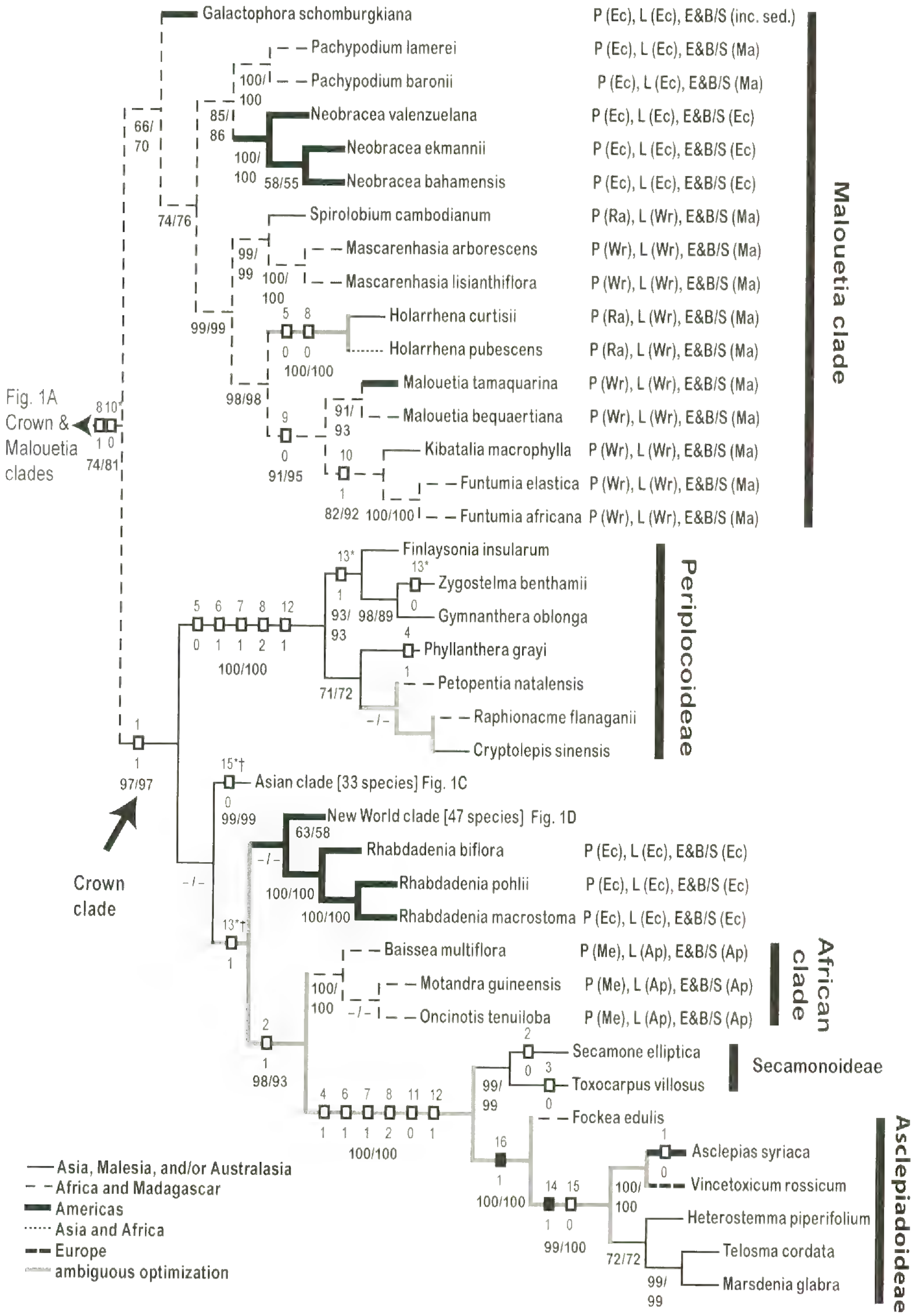

Figure 1B. Crown clade and *Malouetia* clade. See caption for Figure 1A for description.

the genera *Adenium* Roem. & Schult., *Strophanthus* DC., *Isonema*, and *Farquharia* Stapf of the *Nerium* clade (not shown). The chalazal coma of *Kibatalia* G. Don–*Funtumia* Stapf (*Malouetia* clade, Fig. 1B) is unambiguously reconstructed as a parallelism.

WRIGHTIA CLADE (FIG. 1A)

As in all previous analyses (Sennblad & Bremer, 1996, 2002; Sennblad et al., 1998; Potgieter & Albert, 2001), the *Wrightia* clade is strongly supported as

Figure 1C. Asian clade. See caption for Figure 1A for description.

Fig. 1B
New World
clade

Figure 1D. New World clade. See caption for Figure 1A for description.

monophyletic (100% BS) and as sister to the rest of the APSA clade (100% BS). The *Wrightia* clade includes three genera, two of which are endemic to Africa: *Stephanostema* (one species) and *Pleioceras* Baill. (five species; Barink, 1984), the latter analyzed here phylogenetically for the first time. The third genus. *Wrightia*, with approximately 25 species, is distributed in Africa, Asia, Malesia, Australasia, and Australia (Ngan, 1965; Forster, 1993; Middleton & Santisuk, 2001; Middleton, 2005b). The three genera have been considered as closely related (Ngan, 1965; Barink, 1984; Sennblad et al., 1998), and all three were included in tribe Wrightieae by Pichon (1950a), Leeuwenberg (1994), and Endress and Bruyns (2000). Inclusion of the two African species of *Wrightia* would be essential to test fully its monophyly relative to the African *Stephanostema* and *Pleioceras*.

Like the analysis of Sennblad et al. (1998), the present analysis does not identify any unambiguous synapomorphies for the *Wrightia* clade. The rest of the APSA clade can be easily distinguished from this group by the presence of dextrorsely contorted corollas (char. 3, 1) and of a micropylar coma (char. 9, 1). The contrasting character states are reconstructed as plesiomorphic homologues shared by the *Wrightia* clade and Rauvolfioideae. There are two independent reversals to sinistrorse corollas in the APSA clade, one in the genus *Parameria* Benth. (Asian clade; Fig. 1C) and one in *Toxocarpus* Wight & Arn. (Secamonoideae: Fig. 1B). Secondary loss of a micropylar coma is a synapomorphy for *Malouetia–Kibatalia–Funtumia* (*Malouetia* clade; Fig. 1B).

NERIUM CLADE (FIG. 1A)

The *Nerium* clade is resolved as monophyletic in the strict consensus tree but not supported by bootstrap resampling. Previous analyses, which sampled *Nerium*, *Adenium*, and *Strophanthus* (Sennblad et al., 1998; Sennblad & Bremer, 2002) or *Nerium*, *Strophanthus*, and *Isonema* (Potgieter & Albert, 2001), have found a monophyletic *Nerium* clade with weak support (Sennblad et al., 1998) or an unresolved polytomy in the unweighted strict consensus (Potgieter & Albert, 2001; Sennblad & Bremer, 2002). Sennblad et al. (1998) identified the presence of slits in the corolla tube and elongate apical connective appendages on the anthers as synapomorphies of the *Nerium* clade, although the latter are variably present or absent in *Strophanthus* and absent from *Isonema*, *Alafia*, and *Farquharia*. The present study does not identify any synapomorphies for the *Nerium* clade, but it can be diagnosed by the combination of dextrorsely contorted corollas, presence of a micropylar coma, and absence of a nectary around the ovary (char. 8, 0). Seeds of the genera in the *Nerium* clade either have both micropylar and chalazal comas (*Adenium*, *Strophanthus*, *Isonema*, *Farquharia*) or a micropylar coma only (*Nerium*, *Alafia*). Homology of chalazal comas in the *Nerium* clade is ambiguously optimized.

Both Pichon (1950a) and Leeuwenberg (1994) included all taxa of the *Nerium* clade in tribe Wrightieae, with the exception of *Isonema*, which Leeuwenberg transferred to Echiteae. Endress and Bruyns (2000) included all these genera in Wrightieae except *Alafia* and *Farquharia*, which they included in Malouetieae based on the structure of the style-head (Table 2). *Alafia* and *Farquharia*, however, lack the unambiguously reconstructed synapomorphy of the *Malouetia*–crown clades, a nectary around the ovary (char. 8, 1). Furthermore, *Alafia* and *Farquharia* are lianas while all other genera of Malouetieae sensu Endress and Bruyns and of the *Malouetia* clade (Fig. 1B) are exclusively trees and shrubs (with the exception of some individuals of *Galactophora schomburgkiana* Woodson).

The *Nerium* clade is divided into two well-supported subclades, *Nerium–Adenium* (95% BS) and *Strophanthus–Isonema–Alafia–Farquharia* (99% BS). *Nerium* and *Adenium* have been considered closely related to one another on the basis of similar floral morphology, particularly their extremely long, pubescent anther connectives (Pagen, 1987). Dependent growth form (char. 1, 1) is ambiguously optimized as a synapomorphy for the second subclade with a reversal to the self-supporting habit in *Strophanthus boivinii* Baill. (a Madagascan endemic that has been treated as a monotypic segregate genus, *Roupellina* (Baill.) Pichon) and with variable growth form in *Strophanthus preussii* Engl. & Pax (Beentje, 1982). An equally parsimonious reconstruction shows two independent origins of a dependent growth form in this group, one in *Strophanthus caudatus* (L.) Kurz and another in the common ancestor of *Isonema*, *Alafia*, and *Farquharia* (not shown). More extensive sampling within *Strophanthus* and a better understanding of growth forms within this genus are necessary to elucidate evolutionary patterns of this character and to test further the status of *Roupellina* and a second generic segregate of *Strophanthus*, *Christya* Ward & Harv. (not sampled here).

Alafia and the monotypic *Farquharia* have been previously suggested to be closely related (Leeuwenberg, 1997), but Zwetsloot (1981) considered the latter more closely related to *Strophanthus*. *Alafia* and *Farquharia* are resolved as a clade (56% BS), with *Farquharia* (readily distinguished by the presence of a chalazal coma [char. 10, 1] and of colleters along the petiole [char. 2, 1]) nested in a paraphyletic *Alafia*. Denser sampling of the 23 currently recognized

species of *Alafia* is necessary to further test its monophyly and to determine the status of *Farquharia*.

MALOUETIA CLADE (FIG. 1B)

Both the *Malouetia* clade (70% BS) and its sister group relationship with the crown clade (81% BS) are moderately supported. Previous analyses also resolved a *Malouetia* clade comprising *Mascarenhasia* A. DC., *Funtumia*, and *Holarrhena* (Potgieter & Albert, 2001), these three plus *Pachypodium* Lindl. (Semblad et al., 1998), or these four genera plus *Kibatalia* (Semblad & Bremer, 2002). The position of *Galactophora* Woodson in the *Malouetia* clade is a novel finding of the present study; it was previously part of a large polytomy in the APSA clade (Potgieter & Albert, 2001). Many of the genera of the *Malouetia* clade have been suggested to be closely related to each other on the basis of morphology, e.g., *Malouetia*, *Mascarenhasia*, *Kibatalia*, and *Funtumia* (Van der Ploeg, 1985). Semblad et al. (1998) identified the presence of calcium oxalate packets in the anther stomium and the absence of vertical ridges on the style-head opposite the anthers as synapomorphies for this clade. While the present study does not identify any synapomorphies for the *Malouetia* clade, it is readily diagnosed by the presence of synapomorphies shared with the crown clade (presence of a nectary [char. 8, 1], secondarily lost in *Holarrhena*, and absence of a chalazal coma [char. 10, 0], regained in *Kibatalia–Funtumia*) and the plesiomorphic presence of the self-supporting growth form (char. 1, 0). All taxa in the *Malouetia* clade are exclusively trees or shrubs except for *Galactophora schomburgkiana*, which has both vining and shrubby varieties (Monachino, 1958; Zarucchi et al., 1995). The dependent habit is also very rare in *Galactophora*: most of the six currently recognized species are exclusively shrubs (Zarucchi et al., 1995; Morales, 2005).

All taxa of the *Malouetia* clade except *Galactophora* have cylindrical or fusiform style-heads and lack a basal collar (Table 2). The style-head of *Galactophora* has been difficult to characterize because the flowers are highly distorted by pressing. It was described and illustrated by Woodson (1936) as having five ribs and by Pichon (1950a) as having a basal collar. Simões et al. (2004) reported that it has five basal ribs distinct from the rest of the style-head. In the recent revision of the genus, the style-head is described as having five longitudinal ribs at the base, and these are clearly visible in the illustrations for each species (Morales, 2005).

Of the genera included in the *Malouetia* clade, Pichon (1950b) placed *Holarrhena* and *Spirolobium*, along with *Carruthersia* (unsampled here), in subfamily Plumerioideae (Rauvolfioideae) as subtribe Holarrheninae of tribe Alstonieae on the basis of a misinterpretation that the flowers lacked a gynostegium (Pichon, 1950b). He placed *Mascarenhasia*, *Malouetia*, *Kibatalia*, and *Funtumia* in Wrightieae and *Galactophora*, *Pachypodium*, and *Neobracea* in the Echiteae on the basis of their retinacle types (Table 2). The visor-like retinacle could be reconstructed as a symplesiomorphy shared by *Galactophora*, *Pachypodium*, and *Neobracea*, with a brush retinacle evolving in the common ancestor of the rest of the *Malouetia* clade, although it would be equally parsimonious to regard the visor-like retinacle as having evolved independently in *Galactophora* and in the common ancestor of *Pachypodium* and *Neobracea*, with the brush retinacle a symplesiomorphy shared by the rest of the *Malouetia* clade with the *Nerium* and *Wrightia* clades.

Leeuwenberg (1994) transferred *Spirolobium*, *Holarrhena*, and *Carruthersia* to Wrightieae on the basis of floral and pollen morphology and secondary chemistry as reported by Endress et al. (1990). He retained *Galactophora*, *Pachypodium*, and *Neobracea* in Echiteae.

Endress and Bruyns (2000) classified most of the genera of the *Malouetia* clade in their tribe Malouetieae, but they retained *Neobracea* in Echiteae, following Pichon and Leeuwenberg. They transferred *Galactophora* to Mesechiteae based on its style-head with five basal ribs (Table 2). Simões et al. (2004) found that *Galactophora* grouped with their outgroup taxa, *Pachypodium* and *Mascarenhasia*, and lacked most of the synapomorphies that they identified for Mesechiteae (see below). They treated *Galactophora* as a genus incertae sedis.

Intergeneric relationships within the *Malouetia* clade are well resolved and moderately to well supported. Loss of a micropylar coma is resolved as a synapomorphy for *Malouetia–Kibatalia–Funtumia* (char. 9, 0) and secondary gain of a chalazal coma as a synapomorphy for *Kibatalia–Funtumia* (char. 10, 1). Seeds of African species of *Malouetia* are pilose on both the micropylar and chalazal ends (Van der Ploeg, 1985); those of American taxa can be glabrous or pubescent, but the trichomes are not restricted to the chalazal and micropylar ends (Zarucchi et al., 1995). Our sampling of *Malouetia* is inadequate to reconstruct the evolution of seed pubescence and to judge its homology with comas in other genera. *Spirolobium* and *Holarrhena* have an unusual retinacle, inserted on the filament well below the anther (Endress et al., 1990). In the current analysis, this feature is reconstructed as a parallelism, as *Spirolobium* is sister to *Mascarenhasia* and *Holarrhena* is sister to *Malouetia–Kibatalia–Funtumia*. *Holarrhena*

has two synapomorphies, loss of lignification in the anthers (char. 5, 0) and loss of a nectary disk (char. 8, 0). This reversion to a rauvolfioid-like flower may be correlated with a change in pollinators or pollination mechanism.

CROWN CLADE (FIG. 1B)

The crown clade is strongly supported as monophyletic (97% BS). A congruent clade was resolved in several previous studies (Sennblad & Bremer, 1996, 2002; Sennblad et al., 1998). Sennblad et al. (1998) identified anthers with lignified tissue adaxial to the vascular strand and adnation of the anther thecae bases to the style-head as synapomorphies of the crown clade, but this was based on a very small sample, only seven genera of crown-clade Apocynoideae and one genus each of Secamonoideae and Periploceae.

The present study identifies the presence of dependent growth form (char 1, 1) as the only synapomorphy for the crown clade, although there are frequent reversals to self-supporting growth forms in taxa inhabiting more temperate and xeric habitats, e.g., *Apocynum*, *Asclepias*, *Cycladenia* Benth., *Rhodocalyx* Müll. Arg., and many species of *Mandevilla* (Fig. 1B–D), and convergent evolution of dependent growth in the *Nerium* clade (Fig. 1A), *Galactophora* (*Malouetia* clade), and in *Pleioceras* (*Wrightia* clade). Lahaye et al. (2005) identified the self-supporting growth form as plesiomorphic and the dependent growth form as apomorphic in the APSA clade, but with their much sparser sample of Apocynoideae, they could not identify at what level of the phylogeny dependent growth is apomorphic. Only Lý (1986) emphasized growth form in his tribal classification of Apocynoideae, recognizing three tribes of predominantly or entirely self-supporting genera and three of lianas, although he grouped genera with plesiomorphic and secondarily derived self-supporting growth forms together into his tribe Apocyneae.

Pichon (1950a) classified Apocynoideae genera of the crown clade in tribes Apocyneae, Echiteae, and Mesechiteae, except *Beaumontia* Wall., *Vallaris* Burm. f., *Pottsia* Hook. & Arn., and *Aganosma marginata* (Roxb.) G. Don (as part of *Amphineurion* (A. DC.) Pichon), which he included in Wrightieae. Leeuwenberg (1994) followed Pichon in his placement of *Beaumontia* and *Vallaris* but transferred *Pottsia* and *A. marginata* to Echiteae, thus classifying the vast majority of crown clade Apocynoideae in either Apocyneae or Echiteae (Fig. 1C, D). Endress and Bruyns (2000) transferred *Beaumontia* and *Vallaris* to Apocyneae, including all crown clade Apocynoideae in their tribes Apocyneae, Echiteae, and Mesechiteae (Fig. 1C, D).

ASIAN CLADE (FIG. 1C)

The Asian clade is strongly supported as monophyletic (99% BS). All its members are endemic to tropical and subtropical Asia, Malesia, or Australasia, except *Apocynum*, which ranges across temperate Asia, southern Europe, and North America (Woodson, 1930). Significantly, all Asian species of *Trachelospermum* Lem. are part of the Asian clade, whereas the American species, *T. difforme* (Walter) A. Gray, is part of the New World clade (Fig. 1D, see discussion below). In previous phylogenetic studies, only Potgieter and Albert (2001) and Simões et al. (2004) resolved a monophyletic Asian clade. The present study does not identify any synapomorphies for the Asian clade.

Pichon (1950a) scattered taxa of the Asian clade among all four of his tribes, and Leeuwenberg (1994) likewise dispersed genera of the Asian clade among all three of the tribes he recognized.

Endress and Bruyns (2000) included all genera of the Asian clade in Apocyneae except *Pottsia*, which they left in Echiteae. This reflects the incorporation of the results of molecular analyses that supported the monophyly of morphologically divergent taxa. They also incorporated additional morphological characters, such as a very short stamen–corolla tube and a fusiform style-head without a basal collar (Endress & Bruyns, 2000; Table 2). The Asian clade is polymorphic for both of these characters, however. Relatively elongate stamen–corolla tubes (i.e., the congenitally fused part of the tube below the insertion of the stamens) are present in *Pottsia* and some species of *Trachelospermum* and *Epigynum* Wight, and basal collars are found in *Amalocalyx* Pierre, *Epigynum*, and *Sindechites* Oliv. (Middleton, 2005a, in press).

Relationships within the Asian clade are well resolved but with varying levels of support. There are a number of implications for generic delimitations (see Appendix 4 for a list of genera resurrected as a result of this study). *Micrechites* Miq., recently synonymized with *Ichnocarpus* R. Br. (Forster, 1992; Middleton, 1994) and represented here by *I. polyanthus* (Blume) P. I. Forst., *I. rhombifolius* (Markgr.) D. J. Middleton, and *I. serpyllifolius* (Blume) P. I. Forst., is well separated from *Ichnocarpus* s. str. (*I. frutescens* (L.) W. T. Aiton). *Aganosma marginata* (Roxb.) G. Don does not form a monophyletic group with the rest of the genus and should be removed to *Amphineurion* (A. DC.) Pichon as was suggested by Pichon (1950a). Both *Micrechites* and *Amphineurion* can be defined by clear

suites of morphological characters not considered as sufficiently diagnostic by recent workers. Middleton (2006) has now reinstated both genera. *Sindechites* is likewise resolved as polyphyletic, with *S. henryi* Oliver strongly supported as sister to *Aganosma marginata* (98% BS) and *S. chinensis* (Merr.) Markgr. & Tsiang moderately supported (62% BS) as sister to a large clade that includes *Urceola* Roxb. and *Epigynum*. The circumscription of *Urceola* was recently much expanded (Middleton, 1995, 1996b); the present topology indicates that it may need to be further expanded to encompass *Parameria*, regarded by Middleton (1995) as closely related. *Epigynum* is also resolved as paraphyletic, but without bootstrap support.

Apocynum is well nested within the Asian clade and strongly supported as sister to *Cleghornia* Wight (99% BS), confirming the hypothesis of Nilsson et al. (1993) that the tetrads (char. 12, 1) in this genus are convergent with those of Periplocoideae (Fig. 1B, C).

NEW WORLD CLADE (FIG. 1D)

The New World clade is weakly supported (58% BS). Previous studies with broad sampling among members of the APSA clade have not resolved a similar clade (Sennblad et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002), but Simões et al. (2004) found a congruent clade with much narrower sampling. All taxa in our New World clade are endemic to the Americas except for the two predominantly Australasian genera *Artia* Guillaumin and *Parsonsia* R. Br.: *Parsonsia* also extends into tropical and subtropical Asia (Boiteau, 1981; Williams, 1996; Middleton, 1997; Li & Huang, 1998; Heads & de Lange, 1999). The present study does not identify any synapomorphies for the New World clade.

Pichon (1950a) included most of the genera of the New World clade in Echiteae. He placed *Odontadenia* Benth. and *Secondatia* A. DC. in Apocyneae, and *Elytropus* Müll. Arg., *Forsteronia* G. Mey., *Mesechites* Müll. Arg. (including *Allomarkgrafia* Woodson [Pichon, 1950a]), and *Mandevilla* (including *Macrosiphonia* Müll. Arg. [Pichon, 1948a]) in Mesechiteae. Leeuwenberg (1994) included the entire New World clade in his Echiteae, except *Forsteronia*, which he transferred to Apocyneae.

Endress and Bruyns (2000) largely concurred with Pichon (1950a) in their classification of most taxa of the New World clade, although they followed Leeuwenberg (1994) in retaining *Forsteronia* in Apocyneae, where they also moved *Elytropus* and *Trachelospermum* (including *T. difforme*). Finally, they transferred *Secondatia* and *Galactophora* to Mesechiteae.

Simões et al. (2004, 2006, 2007a) concurred with Pichon's exclusion of *Galactophora* and *Secondatia* from Mesechiteae and with the inclusion of *Macrosiphonia* in *Mandevilla*. They identified the distinctive retinacle, formed by cellular fusion rather than adhesion via trichomes (char. 6, 1), the five-ribbed style-head, truncate basal anther appendages, and presence of foliar colleters (char. 2, 1) as synapomorphies of Mesechiteae. They also resolved *Secondatia* and *Odontadenia* as sister taxa, returning the former to Apocyneae.

Morales (2003) found the "spool-shaped" style-head of *Secondatia* consistent with tribe Echiteae, a conclusion congruent with its position in the New World clade but outside the *Mesechites* subclade.

The resolution of *Elytropus* as sister to the *Mesechites* subclade, albeit without bootstrap support, is intriguing given Pichon's inclusion of it in Mesechiteae (Pichon, 1950a). However, this unusual monotypic genus, endemic to the temperate rainforests of Chile and Argentina (Ezcurra, 1999), has none of the synapomorphies of Mesechiteae. It lacks foliar colleters and has acute basal anther appendages, a retinacle of agglutinated trichomes, and a pentagonal style-head in cross section.

Many relationships among the major clades within the New World clade are not resolved or are unsupported, but the diversity of retinacle structures and style-head shapes in the group points to previously unsuspected lability of these characters.

The present study resolves *Forsteronia* as polyphyletic, with *F. corymbosa* (Jacq.) G. Mey. (not sampled by Simões et al. [2004]) forming a strongly supported clade with *Trachelospermum difforme* (100% BS). Hansen (1985) recognized the distinctiveness of *F. corymbosa* and of three congeners from Central America and the Caribbean, erecting the invalid subgenus *Pinochia* for them. These four species lack foliar colleters, have acute basal anther appendages, and have a basal collar on the style-head; their retinacle has not been studied. The inclusion of these species in *Forsteronia* is probably due to the similar appearance of their small flowers, with almost completely exserted stamens, a condition otherwise quite rare in New World Apocynoideae. The present topology would be congruent either with treating these species as congeneric with *T. difforme* or erecting a new genus for them.

Trachelospermum difforme, endemic to the southeastern United States, was initially described in *Echites* P. Browne and transferred by various authors to *Forsteronia*, *Secondatia*, and the illegitimate *Thyrsanthus* Benth. (see Krings, 2003). Gray (1878: 85) placed it "somewhat dubiously" in the Asian genus *Trachelospermum*, probably based on the bio-

geographic affinity between the southeastern United States and East Asia. Pichon (1948c) discussed the morphological differences between Asian and American *Trachelospermum* and erected the monotypic genus *Thyrsanthella* (Baill.) Pichon for the latter by elevating *Forsteronia* section *Thyrsanthella* Baill. to generic rank. However, *T. difforme* has been retained in *Trachelospermum* in the floristic literature (e.g., Rosatti, 1989), a position no longer tenable given the results of the present study (Fig. 1C, D).

Our results also have implications for a number of other generic circumscriptions. Morales (1998) synonymized *Salpinctes* Woodson (here resolved as sister to *Pentalinon* Voigt–*Angadenia* Miers [100% BS]) with *Mandevilla*, although it has none of the synapomorphies of Mesechiteae, lacking foliar colleters and possessing acute basal anther appendages, a visor retinacle, and a style-head with a basal collar. Combined with its position in the present analysis, this is strong evidence for recognizing it as a distinct genus.

Prestonia R. Br. is resolved as polyphyletic, with most of the species sampled here grouping with *Artia* and *Parsonsia* (100% BS), whereas *Prestonia riedelii* (Müll. Arg.) Markgr. is sister to *Rhodocalyx* (77% BS) in a separate clade. Ezcurra et al. (1992) pointed out the many similarities between *Prestonia riedelii* and the monotypic *Rhodocalyx*, which led Morales (1999b) to transfer *Rhodocalyx* to *Prestonia*. Our results highlight the need to reappraise *Prestonia*, since its only diagnostic character is the presence of an annulus in the throat of the corolla (Woodson, 1936; Morales, 1997b). *Laubertia* A. DC. has also been associated with *Prestonia* on the basis of this feature (Woodson, 1931; Morales, 2002) but occupies a position separate from all *Prestonia* species in our analysis.

The Australasian genera *Parsonsia* and *Artia* are nested within a paraphyletic (albeit unsupported) grade of species of the predominantly South American *Prestonia*. These three genera share the presence of syncarpous fruits (found in all *Parsonsia* and *Artia* and some *Prestonia* species) and clear latex. A number of additional taxa will need to be sampled to draw firmer conclusions about the closest relatives of *Artia* and *Parsonsia* in the New World: *Hylaea* J. F. Morales (Morales, 1999a), *Thoreauea* J. K. Williams (Williams, 2002), and *Thenardia* Kunth (Williams, 1998, 1995) from the New World, *Ecua* D. J. Middleton (Middleton, 1996a) from Malesia, as well as a larger sample of *Prestonia* and *Parsonsia* species. Our results confirm Middleton's (1997) decision to expand the circumscription of *Parsonsia* to include the monotypic *Delphyodon* K. Schum. (*P. oligantha* (K. Schum.) D. J. Middleton in Fig. 1D). *Artia*, endemic to New Caledonia after the removal of the Asian species to *Parsonsia* (see Middleton, 1997), bears many similarities to the latter genus. In our results, *Artia* is sister to a monophyletic *Parsonsia* (92% BS), but the sampling in *Parsonsia*, a genus of ca. 82 species, remains low.

The circumscription of *Echites* has varied enormously (Woodson, 1933, 1936; Morales, 1997a). Most recently, Morales and Williams (2004) erected a new genus, *Allotoonia* J. F. Morales & J. K. Williams, for the five species of *Echites* subgenus *Pseudechites* Woodson and restricted *Echites* s. str. to the four species of subgenus *Echites*, a decision based on a cladistic analysis of morphological characters that resolved the two subgenera of *Echites* as polyphyletic with subgenus *Echites* sister to *Fernaldia* Woodson and subgenus *Pseudechites* sister to *Thenardia* (not sampled here; Williams, 2004). The present study supports inclusion of *Allotoonia* in *Echites* s.l. and the expansion of the circumscription of *Echites* to include *Fernaldia* with high bootstrap values (99%–100% BS). The affinity between *Fernaldia* and *Echites* and the paucity of characters separating the two genera has been noted by Williams (2004). Characters used to separate *Echites* s. str. and *Allotoonia* include visibility of tertiary leaf venation, inflorescence branching, corolla lobe shape, and latex color, milky in *Echites* s. str. versus clear in *Allotoonia* (Morales & Williams, 2004). However, the latex of *E. umbellatus* Jacq., which groups with the species segregated into *Allotoonia*, has been reported as both clear and milky on herbarium labels, and living plants from the Bahamas and southern Florida were always found to have clear latex (E. Fried, B. Rathke, S. Zona, pers. comm.).

RHABDADENIA (FIG. 1B)

Our analysis places *Rhabdadenia*, a Caribbean and South American genus of four species (Ezcurra et al., 1992) in a polytomy with the New World clade and the African clade–Secamonoideae–Asclepiadoideae. This genus has been consistently included in Echiteae, but Pichon (1948b) regarded its retinacle as unusually reduced to a weak longitudinal nonfunctional rib with the anthers adhering to the style-head only by the bases of the anther thecae. However, there is a tuft of trichomes at the base of the anther that appears to be weakly attached to the style-head, rather similar to a brush retinacle.

AFRICAN CLADE (FIG. 1B)

The African clade, composed of *Baissea* (18 species)–*Motandra* (3 species)–*Oncinotis* (7 species),

is strongly supported as monophyletic (100% BS). Its greatest species diversity is in tropical West Africa, but one species of *Oncinotis* is endemic to Madagascar (Markgraf, 1976; De Kruif, 1983, 1985; Van Dilst, 1995). These genera have unique branched trichomes and leaves with domatia, which are likely synapomorphies (Omino, 1996).

Pichon (1950a) included these genera in Mesechiteae as subtribe Baisseinae based on his misinterpretation of their retinacles as glabrous facets (Table 2) and on the presence of colleters on the leaves. Nilsson et al. (1993) investigated floral anatomy of *Baissea* and concluded that the retinacle is formed via adhesion by trichomes. We have confirmed this for representatives of all three genera of the African clade. Both Leeuwenberg (1994) and Endress and Bruyns (2000) included these three genera in their Apocyneae on the basis of several characters that occur frequently in the Asian clade, including the very short stamen–corolla tube, half-inferior ovaries, and collarless style-head. These characters are also present in Periplocoideae, Secamonoideae, and Asclepiadoideae and may be symplesiomorphies for the crown clade. *Baissea* has frequently been associated with the Asian *Cleghornia*, which Bentham (1876) treated as a synonym (see Macfarlane, 1933; Xu, 1988).

AFRICAN CLADE SECAMONOIDEAE–ASCLEPIADOIDEAE (FIG. 1B)

The African clade is strongly supported as sister to Secamonoideae–Asclepiadoideae (93% BS), a result corroborated by Lahaye et al. (2007) and Simões et al. (2007b). Various hypotheses of relationships between *Baissea* and these two subfamilies have been resolved in previous analyses, but always without support (Sennblad et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002).

The presence of colleters on the leaves (char. 2, 1) is unambiguously optimized as a synapomorphy for the African clade and Secamonoideae–Asclepiadoideae. However, in the African clade, colleters are predominantly distributed along the adaxial surface of the petiole and occasionally extend onto the base of the lamina, whereas in Secamonoideae and Asclepiadoideae, they are on the base of the lamina and do not extend along the petiole.

Prior to the study of Sennblad et al. (1998), only Macfarlane (1933) had suggested that *Baissea* and *Oncinotis* might be most closely related to Secamonoideae–Asclepiadoideae, although he did not identify colleters on the petiole as diagnostic for this group. In fact, the characters he cited in his argument for these genera as ancestral to Secamonoideae–Asclepiadoideae are mostly widespread in the crown clade or were misinterpreted by him. Macfarlane probably reached this conclusion because of his interest in geographic distributions and centers of origin. Most of the approximately 200 species of Secamonoideae are endemic to Africa and Madagascar (Klackenberg, 1992).

EVOLUTION OF COMPLEX POLLINATION MECHANISMS (FIG. 1B)

Asclepiadaceae (Periplocoideae and Secamonoideae–Asclepiadoideae) are here resolved as polyphyletic, although there is no bootstrap support for the node that precludes a paraphyletic Asclepiadaceae. The characters that have been interpreted as evidence for their sister group relationship (retinacle formed by cellular fusion [char. 6, 1], presence of a translator [char. 7, 1], nectar secretion from staminal feet or a fused basal tube [char. 8, 2], pollen dispersed in tetrads [char. 12, 1]) are reconstructed as parallelisms. The presence of pollinia (char. 13, 1) and of a fused basal tube (char. 4, 1) are reconstructed as parallelisms for Secamonoideae–Asclepiadoideae and certain genera of Periplocoideae. The most recent common ancestor (MRCA) of the African clade–Secamonoideae–Apocynoideae is reconstructed as having pollen in aperturate monads, a foamy style-head adhesive that is not molded into translators, no fused basal tube, and a nectary disk around the ovary, whereas the MRCA of Secamonoideae–Asclepiadoideae is reconstructed as having inaperturate pollen aggregated into four pollinia, translators, and anthers inserted on a fused basal tube with the tissue of alternistaminal sectors of the tube nectariferous. In other words, there are no extant representatives of lineages that evolved along the branch between the MRCA of the African clade–Secamonoideae–Asclepiadoideae (with flowers that are reconstructed as indistinguishable from most other crown clade Apocynoideae) and the MRCA of Secamonoideae–Asclepiadoideae (with flowers that are reconstructed as indistinguishable from modern Secamonoideae).

Simões et al. (2007b), using a much smaller sample of APSA clade taxa, found weak support for a paraphyletic Asclepiadaceae. According to their topology, a retinacle formed by cellular fusion (char. 6, 1), presence of a translator (char. 7, 1), nectar secretion from staminal feet or a fused basal tube (char. 8, 2), and pollen dispersed in tetrads (char. 12, 1) could be reconstructed with equal parsimony as parallelisms or as symplesiomorphies shared by Periplocoideae and Secamonoideae–Asclepiadoideae. If the latter reconstruction is preferred, these characters were secondarily lost in the African clade lineage.

The fossil record yields no additional clues. Konzalova (1981) described fossil massulae, *Asclepia-*

diopollis chebensis, from strata of unknown but suspected Paleogene age from Czechoslovakia. The massulae are aggregates of four tetrahedral to polygonal tetrads with a faintly granular exine and one to three indistinct pores per pollen grain. Although Konzalova (1981) interpreted *A. chebensis* as representing a taxon transitional between Periplocoideae and Secamonoideae, the fossils match quite well the pollen morphology of pollinia-bearing Periplocoideae (represented here by *Gymnanthera* R. Br. and *Finlaysonia* Wall. [Fig. 1B]), which tend to have highly reduced pores in the outer walls of the pollinia (Verhoeven & Venter, 1998, 2001).

BIOGEOGRAPHY (FIG. 1A–D)

Our results demonstrate a stronger correlation between phylogeny and geographic distribution in the APSA clade than suggested by previous classifications of Apocynoideae. The *Wrightia* and *Nerium* clades are exclusively Old World (Fig. 1A). Within the crown clade, Apocynoideae are divided into five geographically structured lineages: the Asian clade (with one New World subclade; Fig. 1C), the New World clade (with one Australasian subclade; Fig. 1D), the New World *Rhabdadenia* (Fig. 1B), and the African clade (Fig. 1B). Two other crown clade lineages, Periplocoideae and Secamonoideae, are exclusively Old World (Fig. 1B). Only the *Malouetia* clade (Fig. 1B) and Asclepiadoideae (Fig. 1B) have multiple subclades with representatives in both the New and Old Worlds. The *Malouetia* clade has two subclades with sister taxa in Africa and the New World: *Neobracea* (Caribbean)–*Pachypodium* (southern Africa and Madagascar) and *Malouetia* (distributed in tropical West Africa and Central and South America; Fig. 1B). *Galactophora*, sister to the rest of the *Malouetia* clade, is endemic to tepuis and white sand areas of the Guyana Shield in South America (Zarucchi et al., 1995). Three subclades of Asclepiadoideae have both Old and New World representatives: *Cynanchum* L., *Marsdenia*, and the subtribe Asclepiadinae (Rapini et al., 2003); all other New World Asclepiadoideae are part of the exclusively New World Metastelmatinae, Oxypetalinae, and Gonolobinae (MOG) clade (Rapini et al., 2003; Liede-Schumann et al., 2005).

The fossil record of the APSA clade is very poor and does not aid in reconstructing the past geographical distributions of its subclades. Only Periplocoideae and *Apocynum* (Asian clade) are known from unambiguous fossils. Fossil pollen of two periplocoid genera, *Tacazzea* Decne. and *Periploca* L., has been reported from the Oligocene and lower Miocene from Cameroon and the Miocene from Eurasia, respectively. Fossil pollen of *Apocynum* has been reported from the upper Miocene from Spain (Muller, 1981). These occurrences are within the current ranges of these genera. Fossil comose seeds from middle to late Eocene North America and middle Eocene to early Oligocene Europe (Manchester, 1999) belong to the APSA clade but cannot be classified with any more taxonomic precision given the absence of diagnostic seed characters for most APSA clade lineages.

CONCLUSIONS

This study represents a major advance in the understanding of phylogenetic relationships within the APSA clade, placing representatives of 59 of 77 currently recognized genera of Apocynoideae in context with exemplars of Periplocoideae, Secamonoideae, and Asclepiadoideae (Fig. 1A, B). The Apocynoideae and their tribes are non-monophyletic as delimited in the classifications of Pichon (1950a), Leeuwenberg (1994), and Endress and Bruyns (2000) as amended by Simões et al. (2004; Fig. 1A–D).

We have identified nested sets of synapomorphies that support relationships among the *Wrightia*, *Nerium*, *Malouetia*, and crown clades (Fig. 1A, B). The *Wrightia* clade retains the plesiomorphic states of self-supporting growth form, sinistrorsely contorted corollas, absence of a nectary, and absence of a micropylar coma; the rest of the APSA clade shares the synapomorphies of dextrorsely contorted corollas and presence of a micropylar coma. The sister relationship between the *Malouetia* clade and the crown clade is supported by the shared presence of a nectary. The crown clade lineages share the synapomorphic presence of dependent growth form. Although parallelisms and/or reversals have occurred in all of these characters (Fig. 1A, B), combined with features of the retinacle, style-head, chalazal coma, and foliar colleters, and also with biogeography (Fig. 1A, B), they are sufficient to diagnose each of the seven primary clades of Apocynoideae identified here.

At this point, the paraphyly of Apocynoideae with respect to Periplocoideae, Secamonoideae, and Asclepiadoideae has been corroborated by numerous studies (Sennblad & Bremer, 1996, 2002; Civeyrel et al., 1998; Sennblad et al., 1998; Potgieter & Albert, 2001; Simões et al., 2007b; this study). If the latter three taxa are maintained as subfamilies in a strictly monophyletic classification of Apocynaceae s.l., it would be necessary to divide Apocynoideae into no fewer than seven subfamilies, corresponding to the *Wrightia*, *Nerium*, *Malouetia*, *Rhabdadenia*, Asian, New World, and African clades (Fig. 1A, B). This number could be reduced if *Rhabdadenia* is treated as incertae sedis and/or if Secamonoideae are reduced to

a tribe of Asclepiadoideae. Whether a strictly monophyletic classification of the APSA clade is desirable and how its constituent groups should be circumscribed will be the subject of future papers.

The non-monophyly of Asclepiadaceae and the sister group relationship of the African clade and Secamonoideae–Asclepiadoideae (strongly supported for the first time in three studies published in this symposium volume [Lahaye et al., 2007; Simões et al., 2007b; this study]) have profound implications for our understanding of the evolution of pollination mechanisms in the Apocynaceae. Of the three proposed scenarios, that of MacFarlane (1933) best fits our phylogenetic result (Fig. 1); pollen aggregation and mass transfer have evolved independently multiple times within the APSA clade. Pollen aggregated into tetrads is reconstructed as evolving at least three times independently: in *Apocynum*, Periplocoideae, and the MRCA of Secamonoideae–Asclepiadoideae (Fig. 1B, C). Pollinia are reconstructed as evolving a minimum of two times independently (once in the MRCA of Secamonoideae–Asclepiadoideae and at least once within Periplocoideae; Fig. 1B, C). Other studies indicate that pollinia have arisen more than once within Periplocoideae (Verhoeven & Venter, 2001; Fishbein, 2001; Ionta & Judd, 2007).

The results presented here indicate that we cannot reconstruct a detailed evolutionary transformation series leading from the flowers of Apocynoideae to those of Secamonoideae. The taxa with intermediate morphologies are extinct and not known from the fossil record. However, the paraphyletic Asclepiadaceae recovered by Simões et al. (2007b) with a much sparser sample of the APSA clade leaves open the possibility that Periplocoideae are indeed the intermediate between Apocynoideae and Secamonoideae and that translators and tetrads were secondarily lost in the African clade. The position of Periplocoideae needs to be clarified by incorporating novel sources of data (e.g., nuclear and mitochondrial genes) in a densely sampled analysis of the APSA clade so that the homologies of the highly modified floral structures of Periplocoideae and Secamonoideae–Asclepiadoideae can be interpreted in the context of a well-supported phylogeny. Future research also needs to focus on identification of organismic synapomorphies for most of the clades reconstructed in this study.

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Appendix 1. Voucher data and GenBank accession numbers. Previously published sequences are cited according to the following code: A = Simões et al. (2004); B = Simões et al. (2007b); B* = identified as *Cerbera venenifera* (Poir.) Steud. in Simões et al. (2007b). Subfamilial and tribal classification is according to Endress and Bruyns (2000) as amended by Endress and Stevens (2001) and Simões et al. (2004). Generic classification of Apocynoideae is that of Endress and Bruyns (2000) as amended by Simões et al. (2004, 2006, 2007a) and Morales and Williams (2004). Generic classification of Periplocoideae is according to Venter and Verhoeven (2001).

Subfamily, Tribe	*Genus species* Author	Voucher provenance, *collector number* (Herbarium acronym)	GenBank accession # *trnL* intron and *trnL-trnF* intergenic spacer; *rps16* intron, *rpl16* intron; *matK* and 3′ *trnK* intron
Apocynoideae, Apocyneae	*Aganosma cymosa* (Roxb.) G. Don	Thailand, *D. J. Middleton 243* (A)	EF456144; EF456608; EF456496; EF456274
	Aganosma cymosa (Roxb.) G. Don	Thailand, *D. J. Middleton et al. 1101* (A)	EF456120; EF456605; EF456389; –
	Aganosma marginata (Roxb.) G. Don	Thailand, *D. J. Middleton et al. 2078* (A)	EF456125; EF456544; EF456491; EF456258
	Aganosma schlechteriana H. Lév.	Thailand, *Newman et al. 1069* (A)	EF456126; EF456606; EF456494; EF456259
	Aganosma wallichii G. Don	Thailand, *D. J. Middleton et al. 1424* (A)	EF456145; EF456609; EF456497; –
	Aganosma wallichii G. Don	Thailand, *D. J. Middleton et al. 1459* (A)	EF456127; EF456607; EF456495; EF456260
	Amalocalyx microlobus Pierre ex Spire	Thailand, *D. J. Middleton 211* (A)	EF456128; EF456545; EF456505; EF456261
	Anodendron oblongifolium Hemsl.	Papua New Guinea, *W. Takeuchi 14513* (A)	EF456123; EF456543; EF456419; EF456256
	Anodendron paniculatum A. DC.	Thailand, *D. J. Middleton et al. 3159* (A)	EF456194; EF456618; EF456499; EF456315
	Apocynum androsaemifolium L.	U.S.A., New York, *T. Livshultz 03-32* (BH, GH)	EF456130; EF456546; EF456387; EF456263
	Apocynum cannabinum L.	U.S.A., New York, *T. Livshultz 03-28* (BH)	EF456131; B; B; B
	Baissea multiflora A. DC.	cult., Belgium, Nat. Bot. Gard. Belgium #74-3527, *F. Billiet S3853* (BR)	EF456199; EF456620; EF456451; EF456319
	Beaumontia grandiflora Wall.	cult., Germany, Bot. Gard. Munich, *G. Gerlach & J. Babczinsky 5/06* (M)	EF456184; EF456556; EF456498; EF456306
	Chonemorpha fragrans (Moon) Alston	Thailand, *D. J. Middleton 220* (A)	EF456132; EF456547; EF456489; EF456264
	Cleghornia malaccensis (Hook. f.) King & Gamble	China, *Tsi Zhanhuo 92-387* (MO)	EF456241; EF456622; EF456504; EF456356
	Elytropus chilensis (A. DC.) Müll. Arg.	Chile, *M. Mihoc et al. Herb. Conc. #156934* (CONC)	EF456228; EF456614; EF456445; EF456307
	Epigynum auritum (C. K. Schneid.) Tsiang & P. T. Li	Thailand, *D. J. Middleton et al. 1073* (A)	EF456121; EF456540; EF456503; EF456253
	Epigynum auritum (C. K. Schneid.) Tsiang & P. T. Li	Thailand, *D. J. Middleton et al. 1457* (A)	EF456146; EF456567; EF456507; –
	Epigynum cochinchinense (Pierre) D. J. Middleton	Thailand, *D. J. Middleton 209* (A)	EF456147; EF456568; EF456508; EF456275
	Epigynum cochinchinense (Pierre) D. J. Middleton	Thailand, *D. J. Middleton et al. 928* (A)	EF456133; EF456548; EF456506; EF456265
	Epigynum griffithianum Wight	Thailand, *D. J. Middleton et al. 1934* (A)	EF456227; EF456626; EF456514; EF456344
	Epigynum ridleyi King & Gamble	Indonesia, Kalimantan, *Ambriansyah & Arifin 315* (L)	EF456245; EF456639; EF456515; EF456359
	Ichnocarpus frutescens (L.) W. T. Aiton	Australia, *P. I. Forster 26539* (BRI)	EF456136; EF456552; EF456490; EF456267
	Ichnocarpus polyanthus (Blume) P. I. Forst.	Thailand, *D. J. Middleton et al. 1950* (A)	EF456225; EF456624; EF456511; EF456342
	Ichnocarpus rhombifolius (Markgr.) D. J. Middleton	Papua New Guinea, *W. Takeuchi & Ama 15692* (A)	EF456190; EF456588; EF456471; –
	Ichnocarpus serpyllifolius (Blume) P. I. Forst.	Brunei, *D. J. Middleton et al. 779* (A)	EF456137; EF456553; EF456472; EF456268

Appendix 1. Continued.

Subfamily, Tribe	*Genus species* Author	Voucher provenance, *collector number* (Herbarium acronym)	GenBank accession # *trnL* intron and *trnL-trnF* intergenic spacer; *rps16* intron, *rpl16* intron; *matK* and 3′ *trnK* intron
	Motandra guineensis (Tonn.) A. DC.	Ivory Coast, *Markin & Pascal s.n.* (Z)	EF456210; EF456621; EF456411; EF456333
	Odontadenia lutea (Vell.) Markgr.	Brazil, *L. S. Kinoshita 2002/56* (UEC)	EF456220; A; –; EF456337
	Odontadenia perrottetti (A. DC.) Woodson	French Guiana, *Prévost & Feuillet 3973* (CAY)	EF456140; EF456554; EF456427; EF456272
	Odontadenia puncticulosa (Rich.) Pulle	French Guiana, *Prévost 3713* (CAY)	EF456187; EF456557; EF456488; EF456310
	Oncinotis tenuiloba Stapf	South Africa, *T. Abbott 7707* (Z)	EF456141; B; B; B
	Papuechites aambe (Warb.) Markgr.	Papua New Guinea, *Takeuchi & Ama 16124* (A)	EF456189; EF456587; EF456448; EF456312
	Parameria laevigata (Juss.) Moldenke	Thailand, *D. J. Middleton et al. 2551* (A)	EF456197; EF456598; EF456513; EF456368
	Pottsia laxiflora (Blume) Kuntze	Thailand, *D. J. Middleton et al. 388* (A)	EF456149; EF456570; EF456493; EF456276
	Secondatia densiflora A. DC.	Brazil, *A. Simões et al. 1218* (UEC)	EF456177; EF456564; EF456440; EF456299
	Sindechites chinensis (Merr.) Markgr. & Tsiang	Thailand, *Newman et al. 1034* (A)	EF456151; EF456572; EF456492; EF456278
	Sindechites henrvi Oliv.	China, *Sino-American Guizhou Botanical Expedition 310* (A)	EF456244; EF456638; EF456502; EF456367
	Trachelospermum asiaticum (Sieb. & Zucc.) Nakai	cult., Belgium, Nat. Bot. Gard. Belgium #56-2159, *F. Billiet S218* (BR)	EF456207; EF456595; EF456517; EF456324
	Trachelospermum asiaticum (Sieb. & Zucc.) Nakai	cult., Belgium, Nat. Bot. Gard. Belgium #98-1661-49, *V. Leyman S3857* (BR)	EF456204; EF456604; EF456516; EF456322
	Trachelospermum asiaticum (Sieb. & Zucc.) Nakai	cult., U.S.A., Cornell University, *T. Livshultz 03-25* (A, BH)	EF456155; EF456573; EF456501; EF456281
	Trachelospermum axillare Hook. f.	Thailand, *Suksathan & D. J. Middleton 1852* (A)	EF456156; EF456574; EF456509; EF456282
	Trachelospermum difforme (Walter) A. Gray	cult., U.S.A., Florida, *G. Ionta 460* (FLAS)	EF456171; EF456562; EF456435; EF456293
	Trachelospermum jasminoides (Lindl.) Lem.	cult., U.S.A., Univ. Florida, *G. Ionta 420* (FLAS)	EF456172; EF456578; EF456436; EF456294
	Urceola lucida (Wall. ex G. Don) Benth. ex Kurz	Thailand, *D. J. Middleton et al. 2058* (A)	EF456226; EF456625; EF456512; EF456343
	Urceloa micrantha (Wall. ex G. Don) D. J. Middleton	Thailand, *D. J. Middleton et al. 1003* (A)	EF456157; EF456575; EF456510; EF456283
	Urceola minutiflora (Pierre) D. J. Middleton	Thailand, *D. J. Middleton 193* (A)	EF456124; –; –; –
	Urceola minutiflora (Pierre) D. J. Middleton	Laos, *D. J. Middleton & Lamxay 287* (A)	EF456158; EF456576; EF456385; –
	Urceola minutiflora (Pierre) D. J. Middleton	Laos, *D. J. Middleton & Lamxay 255* (A)	EF456159; –; –; EF456284
	Urceola rosea (Hook. & Arn.) D. J. Middleton	Thailand, *D. J. Middleton et al. 481* (A)	EF456160; EF456603; EF456386; EF456285
	Vallaris solanacea (Roth) Kuntze	Thailand, *D. J. Middleton et al. 1704* (A)	EF456162; EF456559; EF456500; EF456286
Apocynoideae, Echiteae	*Allotoonia agglutinata* (Jacq.) J. F. Morales & J. K. Williams	Puerto Rico, *C. M. Taylor 8079* (NY)	EF456232; EF456630; EF456460; EF456348
	Allotoonia woodsoniana (Monach.) J. F. Morales & J. K. Williams	Costa Rica, *F. Morales 8810* (INB)	EF456175; EF456580; EF456403; EF456297

Appendix 1. Continued.

Subfamily, Tribe	*Genus species* Author	Voucher provenance, *collector number* (Herbarium acronym)	GenBank accession # *trnL* intron and *trnL-trnF* intergenic spacer; *rps16* intron, *rpl16* intron; *matK* and 3′ *trnK* intron
	Angadenia berteroi (A. DC.) Miers	Cuba, *S. Vilsson s.n.* (Z)	EF456129; EF456611; EF456416; EF456262
	Angadenia sagraei Miers	Bahamas, *M. Vincent 11459* (GH)	EF456182; EF456613; EF456443; EF456304
	Artia balansae (Baill.) Pichon ex Guillaumin	New Caledonia, *G. McPherson et al. 19257* (MO)	EF456209; EF456534; EF456481; EF456325
	Cycladenia humilis Benth.	U.S.A., California, *B. Smith 822* (Z)	EF456211; EF456558; EF456397; EF456334
	Echites turriger Woodson	Mexico, *B. P. Spencer & J. K. Williams 2* (SHST)	EF456196; EF456590; EF456404; EF456317
	Echites umbellatus Jacq.	Bahamas, *M. Vincent 11468* (GH)	EF456181; EF456584; EF456390; EF456303
	Echites umbellatus Jacq.	Mexico, *R. Orellana 975* (NY)	EF456237; EF456633; –; EF456352
	Fernaldia pandurata (A. DC.) Woodson	Costa Rica, *L. Gomez s.n.* (Z)	EF456191; EF456560; EF456401; EF456313
	Laubertia contorta (M. Martens & Galeotti) Woodson	cult., Sam Houston State University, *J. Williams 2005-1* (SHST)	EF456246; EF456640; EF456469; EF456360
	Neobracea bahamensis (Britton) Britton	Bahamas, *M. Vincent 11466* (GH)	EF456183; EF456585; EF456444; EF456305
	Neobracea ekmannii Urb.	Cuba, *S. Nilsson s.n.* (Z)	EF456176; EF456581; EF456439; EF456298
	Neobracea valenzuelana (A. Rich.) Urb.	Cuba, *S. Nilsson s.n.* (Z)	EF456139; EF456565; EF456426; EF456270
	Parsonsia crebriflora Baill.	New Caledonia, *G. McPherson and J. Munzinger 18220* (MO)	EF456214; EF456535; EF456482; EF456327
	Parsonsia eucalyptophylla F. Muell.	Australia, *P. I. Forster 20118* (BRI)	EF456142; B; B; B
	Parsonsia ferruginea J. B. Williams	Australia, *B. Hyland 16142* (QRS)	EF456219; EF456538; EF456486; EF456335
	Parsonsia flexuosa Baill.	New Caledonia, *G. McPherson & J. Munzinger 18046* (MO)	EF456218; EF456537; EF456485; EF456332
	Parsonsia lata Markgr.	Papua New Guinea, *Takeuchi & Ama 16379* (A)	EF456188; EF456533; EF456428; EF456311
	Parsonsia longiflora Guillaumin	New Caledonia, *G. McPherson & J. Munzinger 18151* (MO)	EF456216; EF456536; EF456483; EF456330
	Parsonsia oligantha (K. Schum.) D. J. Middleton	Papua New Guinea, *W. Takeuchi 14502* (A)	EF456143; EF456532; EF456429; EF456273
	Peltastes isthmicus Woodson	Costa Rica, *M. E. Endress 97-16* (Z)	EF456179; EF456583; EF456441; EF456301
	Peltastes peltatus (Vell.) Woodson	cult., Belgium, Nat. Bot. Gard. Belgium #84-2849, *F. Billiet 83526* (BR)	EF456203; B; B; B
	Pentalinon luteum (L.) B. F. Hansen & Wunderlin	Bahamas, *M. Vincent 11460* (GH)	EF456180; EF456612; EF456442; EF456302
	Prestonia coalita (Vell.) Woodson	Brazil, *L. S. Kinoshita et al. 2000/10* (UEC)	EF456185; EF456586; EF456447; EF456309
	Prestonia lagoensis (Müll. Arg.) Woodson	Paraguay, *E. M. Zardini & L. Guerrero 44456* (AS, MO)	EF456215; EF456601; EF456455; EF456329
	Prestonia mexicana A. DC.	Mexico, *E. Martinez et al. 29350* (NY)	EF456229; EF456627; EF456487; EF456345
	Prestonia portobellensis (Beurl.) Woodson	Mexico, *F. Ventura 21262* (NY)	EF456235; EF456631; EF456462; EF456350

Appendix 1. Continued.

Subfamily, Tribe	*Genus species* Author	Voucher provenance, *collector number* (Herbarium acronym)	GenBank accession # *trnL* intron and *trnL-trnF* intergenic spacer; *rps16* intron, *rpl16* intron; *matK* and 3′ *trnK* intron
	Prestonia quinquangularis (Jacq.) Spreng.	cult., Belgium, Nat. Bot. Gard. Belgium #51-0287, *F. Billiet S205* (BR)	EF456205; B; B; B
	Prestonia riedelii (Müll. Arg.) Markgr.	Brazil, *A. O. Simões 1274* (UEC)	EF456212; A; –; EF456336
	Prestonia riedelii (Müll. Arg.) Markgr.	Bolivia, *Solomon & Escobar 12465* (NY)	EF456233; EF456563; EF456461; –
	Prestonia robusta Rusby	Bolivia, *Nee & Chavez 51515* (NY)	EF456236; EF456632; EF456463; EF456351
	Prestonia tomentosa R. Br.	Paraguay, *E. M. Zardini & W. Cardozo 44964* (AS, MO)	EF456217; EF456602; EF456484; EF456331
	Rhabdadenia biflora (Jacq.) Müll. Arg.	U.S.A., Florida, *S. Zona 616* (FTG)	EF456150; EF456571; EF456388; EF456277
	Rhabdadenia macrostoma (Benth.) Müll. Arg.	Peru, *Smith et al. 776* (NY)	EF456234; EF456599; –; EF456349
	Rhabdadenia pohlii Müll. Arg.	Paraguay, *E. M. Zardini & F. Vieira 41979* (MO, PY)	EF456213; EF456597; EF456480; EF456326
	Rhodocalyx rotundifolius Müll. Arg.	Brazil, *L. S. Kinoshita 2000/66* (UEC)	EF456186; EF456561; EF456446; EF456308
	Salpinctes kalmiifolius Woodson	Venezuela, *Steyermark et al. 124577* (NY)	EF456231; EF456629; EF456459; EF456347
	Stipecoma peltigera (Stadelm.) Müll. Arg.	Brazil, *L. S. Kinoshita s.n.* (UEC)	EF456193; EF456616; EF456478; EF456314
	Temnadenia odorifera (Vell.) J. F. Morales	Brazil, *A. O. Simões & R. B. Singer 1035* (UEC)	EF456238; EF456634; EF456464; EF456353
	Temnadenia violacea (Vell.) Miers	Brazil, *A. O. Simões et al. 144* (UEC)	EF456224; EF456623; EF456479; EF456341
Apocynoideae, Malouetieae	*Alafia barteri* Oliv.	Ghana, *H. H. Schmidt et al. 2037* (MO)	EF456239; EF456635; EF456465; EF456354
	Alafia thouarsii Roem. & Schult.	Madagascar, *G. McPherson et al. 17584* (MO)	EF456242; EF456637; EF456467; EF456357
	Farquharia elliptica (Preuss) Stapf	Ivory Coast, *Chatelain et al. 1456* (MO)	EF456242; EF456637; EF456467; EF456357
	Funtumia africana (Benth.) Stapf	cult., Belgium, Nat. Bot. Gard. Belgium #51-4728, *V. Leyman S3855* (BR)	EF456206; EF456594; EF456415; EF456323
	Funtumia elastica (Preuss) Stapf	cult., Belgium, Nat. Bot. Gard. Belgium #81-0051, *V. Leyman S3927* (BR)	EF456202; B; B; B
	Holarrhena curtisii King & Gamble	Thailand, *D. J. Middleton et al. 2042* (A)	EF456122; EF456541; EF456423; EF456254
	Holarrhena curtisii King & Gamble	Laos, *D. J. Middleton & Lamxay 253* (A)	EF456148; EF456569; EF456417; –
	Holarrhena pubescens (Buch-Ham.) Wall. ex G. Don	Tanzania, *Kuchar & Kidua 25042* (MO)	EF456250; EF456643; EF456475; EF456363
	Holarrhena pubescens (Buch-Ham.) Wall. ex G. Don	Tanzania, *Mwangulango & Kipambe 130* (MO)	EF456249; EF456642; EF456474; EF456362
	Holarrhena pubescens (Buch-Ham.) Wall. ex G. Don	Tanzania, *Muiga et al. 39* (MO)	EF456247; EF456641; EF456473; EF456361
	Holarrhena pubescens (Buch-Ham.) Wall. ex G. Don	Thailand, *D. J. Middleton 244* (A)	EF456135; EF456551; EF456421; EF456271
	Kibatalia macrophylla (Pierre ex Hua) Woodson	Thailand, *D. J. Middleton et al. 1018* (A)	EF456119; EF456542; EF456422; EF456255
	Malouetia bequaertiana Woodson	Cameroun, *J. J. F. E. de Wilde 8405* (MO)	EF456243; EF456617; EF456468; EF456358
	Malouetia tamaquarina (Aubl.) A. DC.	French Guiana, *S. Mori et al. 25313* (NY)	EF456230; EF456628; EF456457; EF456346
	Mascarenhasia arborescens A. DC.	cult., Belgium, Nat. Bot. Gard. Belgium #85-0085, *F. Billiet S3053* (BR)	EF456201; EF456593; EF456453; EF456321

Appendix 1. Continued.

Subfamily, Tribe	*Genus species* Author	Voucher provenance, *collector number* (Herbarium acronym)	GenBank accession # *trnL* intron and *trnL-trnF* intergenic spacer; *rps16* intron, *rpl16* intron; *matK* and 3′ *trnK* intron
	Mascarenhasia lisianthiflora A. DC.	Madagascar, *Schönenberger et al. A147* (UPS)	EF456174; EF456579; EF456438; EF456296
	Pachypodium baronii Costantin & Bois	cult., U.S.A., Cornell University, *T. Livshultz 03-20* (BH)	EF456154; EF456615; EF456449; EF456280
	Pachypodium lamerei Drake	cult., Switzerland, Bot. Gard. Zurich, May 2003, *M. E. Endress s.n.* (Z)	EF456198; EF456619; EF456458; EF456318
	Spirolobium cambodianum Baill.	Thailand, *D. J. Middleton et al. 3181* (A)	EF456195; EF456589; EF456450; EF456316
Apocynoideae, Mesechiteae	*Illomarkgrafia brenesiana* Woodson	Costa Rica, *M. E. Endress 97-06* (Z)	EF456223; A; –; EF456310
	Forsteronia acouci (Aubl.) A. DC.	French Guiana, *Prévost 3720* (CAY)	EF456222; A; –; EF456339
	Forsteronia corymbosa (Jacq.) G. Mey.	Puerto Rico, *E. Santiago 97-6* (Z)	EF456167; EF456555; EF456432; EF456289
	Forsteronia guyanensis Müll. Arg.	French Guiana, *Prévost & Feuillet 3970* (CAY)	EF456134; EF456549; EF456477; EF456266
	Forsteronia velloziana (A. DC.) Woodson	Brazil, *Simões 343* (UEC)	EF456221; A; –; EF456338
	Mandevilla boliviensis (Hook. f.) Woodson	cult., U.S.A., Harvard University, *T. Livshultz 03-35* (GH)	EF456153; EF456550; EF456425; EF456279
	Mandevilla longiflora (Desf.) Pichon	Paraguay, *E. M. Zardini & F. Vieira 41973* (MO, PY)	EF456208; EF456600; EF456454; EF456328
	Mesechites trifidus (Jacq.) Müll. Arg.	Ecuador, *Liede & Meve 3471* (UBT)	EF456138; EF456566; EF456424; EF456269
Apocynoideae, Wrighteae	*Adenium obesum* (Forssk.) Roem. & Schult.	cult., U.S.A., Cornell University, *T. Livshultz 03-21* (BH)	EF456152; B; B; B
	Isonema smeathmannii Roem. & Schult.	cult., Belgium, Nat. Bot. Gard. Belgium #64-0527, *F. Billiet S1964* (BR)	EF456200; EF456592; EF456452; EF456320
	Nerium oleander L.	cult., U.S.A., Univ. Florida, *G. Ionta 416* (FLAS)	EF456173; EF456596; EF456437; EF456295
	Pleioceras barteri Baill.	Ghana, *Jongkind et al. 2131* (MO)	EF456251; EF456524; EF456476; EF456364
	Stephanostema stenocarpum K. Schum.	Tanzania, *W. R. Q. Luke et al. 3754* (MO)	EF456248; B; B; B
	Strophanthus boivinii Baill.	cult., Switzerland, Bot. Gard. Zurich, Dec. 2003, *M. E. Endress s.n.* (Z)	EF456192; B; B; B
	Strophanthus caudatus (L.) Kurz	cult., Thailand, Central Botanical Garden, Saraburi Prov., *D. J. Middleton 245* (BKF)	EF456168; EF456577; EF456456; EF456290
	Strophanthus preussii Engl. & Pax	cult., U.S.A., Cornell University, *T. Livshultz 03-24* (BH)	EF456118; EF456539; EF456418; EF456252
	Wrightia arborea (Dennst.) Mabb.	Thailand, *D. J. Middleton 240* (A)	EF456164; B; B; B
	Wrightia coccinea (Roxb. ex Hornem.) Sims	Thailand, *D. J. Middleton et al. 897* (A)	EF456165; EF456527; EF456430; EF456287
	Wrightia dubia (Sims) Spreng.	Thailand, *D. J. Middleton et al. 1399* (A)	EF456163; EF456526; EF456420; EF456257
	Wrightia lanceolata Kerr	Thailand, *D. J. Middleton et al. 288* (A)	EF456169; EF456528; EF456433; EF456291

Appendix 1. Continued.

Subfamily, Tribe	*Genus species* Author	Voucher provenance, *collector number* (Herbarium acronym)	GenBank accession # *trnL* intron and *trnL-trnF* intergenic spacer; *rps16* intron, *rpl16* intron; *matK* and 3′ *trnK* intron
	Wrightia religiosa (Teijsm. & Binn.) Benth. ex Kurz	Thailand, *D. J. Middleton 2033* (A)	EF456170; EF456530; EF456434; EF456292
	Wrightia sirikitiae D. J. Middleton & Santisuk	Thailand, *D. J. Middleton & Wongprasert 579* (A)	EF456166; EF456531; EF456431; EF456288
Apocynoideae, incertae sedis	*Galactophora schomburgkiana* Woodson	Venezuela, *F. Michelangeli 516* (PORT, VEN)	EF456178; EF456582; EF456406; EF456300
Asclepiadoideae, Asclepiadeae	*Asclepias syriaca* L.	U.S.A., New York, *T. Livshultz 03-33* (BH, GH)	EF456111; B; B; B
	Vincetoxicum rossicum (Kleop.) Barbar.	U.S.A., New York, *T. Livshultz 03-31* (BH, GH)	EF456113; EF456651; EF456413; EF456381
Asclepiadoideae, Ceropegieae	*Heterostemma piperifolium* King & Gamble	Thailand, *D. J. Middleton 194* (A)	EF456110; EF456610; EF456384; EF456380
Asclepiadoideae, Fockeae	*Fockea edulis* K. Schum.	cult., U.S.A., Cornell University, 31 March, 1998, *T. Livshultz s.n.* (BH)	EF456115; EF456653; EF456405; EF456383
Asclepiadoideae, Marsdenieae	*Marsdenia glabra* Costantin	Thailand, *D. J. Middleton et al. 1123* (A)	EF456114; EF456652; EF456414; EF456382
	Telosma cordata (Burm. f.) Merr.	cult., U.S.A., Cornell University, *T. Livshultz 01-33* (BH)	EF456112; B; B; B
Periplocoideae	*Cryptolepis sinensis* (Lour.) Merr.	Thailand, *D. J. Middleton et al. 1016* (A)	EF456104; EF456644; Ef456407; EF456374
	Finlaysonia insularum (King & Gamble) Venter	Thailand, *D. J. Middleton et al. 1164* (A)	EF456105; EF456645; EF456408; EF456375
	Gymnanthera oblonga (Burm. f.) P. S. Green	Australia, *A. Ford 2607* (BRI)	EF456106; EF456646; EF456409; EF456376
	Petopentia natalensis (Schltr.) Bullock	cult., U.S.A., Cornell University, *T. Livshultz 03-2* (BH)	EF456107; B; B; B
	Phyllanthera grayi (P. I. Forst.) Venter	Australia, *P. I. Forster 24232* (BRI)	EF456103; B; B; B
	Raphionacme flanaganii Schltr.	cult., U.S.A., Cornell University, *T. Livshultz 03-23* (BH)	EF456108; EF456647; EF456410; EF456377
	Zygostelma benthamii Baill.	Thailand, *D. J. Middleton et al. 849* (A)	EF456109; EF456648; Ef456399; EF456378
Rauvolfioideae, Alyxieae	*Alyxia grandis* P. I. Forst.	Australia, *D. J. Middleton 703* (A)	EF456089; EF456518; EF456393; EF456369
	Alyxia oblongata Domin	Australia, *D. J. Middleton 702* (A)	EF456090; EF456519; EF456394; EF456370
	Alyxia reinwardtii Blume	Thailand, *D. J. Middleton 172* (A)	EF456091; B; B; B
	Alyxia spicata R. Br.	Australia, *D. J. Middleton 701* (A)	EF456092; EF456521; EF456395; EF456371
	Chilocarpus costatus Miq.	Thailand, *D. J. Middleton et al. 370* (A)	EF456096; B; B; B
	Chilocarpus rostratus Markgr.	Malaysia, Sabah, *S. Davies et al. 99272* (A)	EF456097; EF456523; EF456391; –
	Condylocarpon isthmicum (Vell.) A. DC.	Brazil, *L. S. Kinoshita et al. 98-267* (UEC)	EF456087; B; B; B
	Plectaneia thouarsii Roem. & Schult.	Madagascar, *J. S. Miller et al. 8769* (MO)	EF456102; EF456529; EF456402; EF456373
	Pteralyxia kauaiensis Caum	U.S.A., Hawaii, *D. Lorence 7768* (PTBG, Z)	EF456098; EF456520; EF456470; –
Rauvolfioideae, Carisseae	*Carissa spinarum* L.	Thailand, *Newman et al. 1131* (A)	EF456093; EF456591; EF456400; EF456372
Rauvolfioideae, Plumerieae	*Allamanda schottii* Pohl	cult., U.S.A., Cornell University, *T. Livshultz 03-26* (BH)	EF456099; B; B; B
	Cerbera manghas L.	Thailand, *D. J. Middleton et al. 2047* (A)	EF456094; B; B; B

Appendix 1. Continued.

Subfamily, Tribe	*Genus species* Author	Voucher provenance, *collector number* (Herbarium acronym)	GenBank accession # *trnL* intron and *trnL-trnF* intergenic spacer; *rps16* intron, *rpl16* intron: *matK* and 3′ *trnK* intron
Secamonoideae	*Cerbera manghas* L.	cult., Belgium, Nat. Bot. Gard. Belgium #77-0211, *F. Billiet S526*	EF456101: B*: B*: B*
	Cerbera odollam Gaertn.	Malaysia, Sabah, *Davies et al. 99280* (A)	EF456095: EF456522: EF456392: EF456365
	Himatanthus bracteatus (A. DC.) Woodson	French Guiana. *S. Mori 25799* (NY)	EF456161: EF456525: EF456396: EF456366
	Plumeria cubensis Urb.	cult., U.S.A., Hawaii, Nat. Trop. Bot. Gard. Kauai #86059003. Aug. 2003. *M. E. Endress s.n.* (PTBG)	EF456100: B: B: B: B
	Thevetia peruviana K. Schum.	cult., U.S.A., Univ. Comm., *T. Livshultz 03-34* (GH)	EF456088: B: B: B: –
	Secamone elliptica R. Br.	Thailand, *D. J. Middleton et al. 1162* (A)	EF456116: B: B: B: B
	Toxocarpus villosus (Blume) Decne.	Thailand, *D. J. Middleton et al. 1341* (A)	EF456117: EF456398: EF456379

Appendix 2. Morphological characters, coding, and discussion.

1. **Growth form**: 0 = self-supporting; 1 = dependent. Self-supporting encompasses herbs, shrubs, and trees that hold their branches without assistance from other structures. Dependent encompasses herbaceous or woody vines and lianas that either twine around or lean against other structures to support their branches. While both self-supporting and dependent are emergent properties of potentially non-homologous aspects of plant anatomy and physiology, this is the best possible coding without extensive anatomical and physiological investigation. Species that have been reported both as self-supporting and dependent or as somewhere in between, e.g., semi-twining in field notes of *Steyermark 58220* for *Salpinctes kalmiifolius* Woodson, are coded as polymorphic for this character.

Most Wrightieae and Malouetieae are self-supporting, although some are dependent, e.g., *Alafia* Thouars, *Isonema* R. Br., *Farquharia* Stapf, many species of *Strophanthus* DC. Dependent growth forms predominate in the rest of the APSA clade, although many taxa occurring in more xeric, e.g., *Adenium* Roem. & Schult., or temperate, e.g., *Apocynum* L., *Asclepias* L., climates are self-supporting.

2. **Colleters on adaxial surface of leaf (petiole or lamina)**: 0 = absent; 1 = present. Colleters on the base of the adaxial surface of the leaf lamina are characteristic for almost all species of Asclepiadoideae, some species of Secamonoideae, and most species of tribe Mesechiteae of subfamily Apocynoideae. The genera *Farquharia* (Malouetieae), *Isonema* and *Verium* L. (Wrightieae), *Baissea* A. DC., *Oncinotis* Benth., and *Motandra* A. DC. (Apocyneae) have colleters primarily on the adaxial surface of the petiole, in some species extending to the junction of petiole and lamina and onto the base of the leaf lamina. Given the ambiguity in distinguishing the apex of the petiole and the base of the leaf lamina, colleters anywhere on the adaxial surface of the leaf, superaxillary colleters sensu Pichon (1949), are coded as homologous.

3. **Corolla aestivation**: 0 = sinistrorse; 1 = dextrorse; 2 = valvate. Dextrorse aestivation of corolla lobes in the floral bud is one of the diagnostic characters for the APSA clade, but sinistrorse aestivation occurs in *Wrightia* R. Br., *Stephanostema* K. Schum., *Pleioceras* Baill., *Parameria* Benth., and *Toxocarpus* Wight & Arn. Valvate aestivation is diagnostic for tribe Ceropegieae of Asclepiadoideae and occurs in some genera of Apocynoideae including *Urceola* Roxb. and *Parsonsia* R. Br.

4. **Fused basal tube**: 0 = absent; 1 = present. In Rauvolfioideae and Apocynoideae, the stamens are inserted atop a congenitally fused stamen–corolla tube (Nishino, 1982). In Periplocoideae, the stamens are typically inserted atop five, separate, more or less prominent lobes that often also bear a corona lobe at their apices, abaxial to the stamens. These lobes have been called the basal tube (Kunze, 1990), staminal feet (Endress & Bruyns, 2000), or basal corona lobes (Simões et al., 2007b). In Asclepiadoideae and some Secamonoideae and Periplocoideae, the anthers are borne atop a fused tube of tissue that arises as a solid ring and tightly encircles the gynoecium. In many Asclepiadoideae, corona lobes are also borne on this tube (termed the basal tube by Endress & Bruyns [2000]), abaxial to the anthers. Most workers have interpreted this tube in Asclepiadoideae as fused staminal filaments (Woodson, 1954; Sattler, 1973; Kunze, 1996), unfused or fused only at the very base in Secamonoideae (Safwat, 1962; Kunze, 1996), but Endress and Bruyns (2000) proposed that it is a specialized type of ring corona and that the anthers of

Asclepiadoideae and Secamonoideae are sessile at its apex. We have accepted this latter interpretation for this analysis. Because the topological relationship of the stamen–corolla tube, the staminal feet, and the basal tube to the stamens is the same (i.e., the stamens are inserted atop each of these), we here interpret the latter two as outgrowths of the stamen–corolla tube. We code all taxa except *Phyllanthera* Blume, Secamonoideae, and Asclepiadoideae as lacking a fused basal tube in order to contrast them with the latter three, which are coded as sharing the presence of a fused basal tube.

5. **Lignified anthers**: 0 = absent; 1 = present. Lignification of the anthers is diagnostic for the APSA clade but also characterizes most Tabernaemontaneae (Rauvolfioideae; Fallen, 1986; Simões et al., 2007b). It is absent in most Periplocoideae (Nilsson et al., 1993; Endress & Bruyns, 2000) and in *Holarrhena* R. Br. (Endress et al., 1990) and has been the basis for some workers associating these taxa with Rauvolfioideae rather than with the APSA clade (Schumann, 1895; Wanntorp, 1988).

6. **Retinacle**: 0 = agglutinated trichomes; 1 = cellular fusion. Union between the apex of the gynoecium (the style-head) and the stamens, forming a gynostegium, is a synapomorphy for the APSA clade, present in all included taxa, although the attachment is very weak in some Malouetieae and Wrightieae, e.g., *Holarrhena*, *Nerium*. The region of the stamen by which it attaches to the style-head to form the gynostegium was termed the retinacle by Pichon (1948b) and used by him as the basis of his tribal classification (Table 2). More detailed studies using anatomical sections showed that retinacles are formed either by cellular fusion or by agglutination via trichomes (Nilsson et al., 1993; Kunze, 1996). Retinacles formed by cellular fusion have been found in all Periplocoideae, Secamonoideae, and Asclepiadoideae studied to date. Simões et al. (2004) also found formation of the retinacle via cellular fusion in some of the genera Pichon described as having a retinacle of a glabrous facet (*Allomarkgrafia* Woodson, *Mesechites* Müll. Arg., and *Mandevilla* Lindl.). Conversely, anatomical sectioning has shown that *Elytropus* Müll. Arg., *Baissea*, *Motandra*, and *Oncinotis* (described by Pichon [1948b] as having a glabrous facet retinacle) have retinacles formed by agglutination of trichomes (Nilsson et al., 1993; M. E. Endress, unpublished). Retinacle anatomy has yet to be studied in many genera of Apocynoideae, but we have chosen to include it because it is a potential synapomorphy for Periplocoideae, Secamonoideae, and Asclepiadoideae. We have coded cellular fusion as present in these three subfamilies as well as in *Allomarkgrafia*, *Mesechites*, and *Mandevilla*. *Forsteronia* G. F. W. Mey. is scored as polymorphic for this character following Simões et al. (2004). Genera described as having a glabrous facet retinacle by Pichon (1950a) but not yet studied in anatomical sections (*Parameria* Benth., *Ichnocarpus* R. Br., *Epigynum* Wight, and *Sindechites* Oliv.) are scored as unknown for this character. The rest of Apocynoideae are scored as having a retinacle formed by trichomes, and the outgroup genera are scored as inapplicable.

7. **Pollen transport adhesive**: 0 = a foamy or gel-like adhesive that may harden as the flower desiccates, forming amorphous translators; 1 = five translators each with a hardened corpusculum (Secamonoideae and Asclepiadoideae) or stalk (Periplocoideae) at anthesis, bifid in cross section, secreted in alternistaminal grooves on the style-head, with (most Asclepiadoideae) or without (most Secamonoideae) caudicles or with a basal adhesive pad viscidium and distal spoon (Periplocoideae). Presence of translators was the diagnostic character used by Brown (1810) to separate Asclepiadaceae from Apocynaceae s. str. (Endress, 2004). However, amorphous translators, five discrete, hardened masses of adhesive positioned alternistaminally on the style-head, have been reported in *Apocynum* and *Forsteronia* (Nilsson et al., 1993). These observations were made on alcohol-preserved and dehydrated flowers. In fresh, unprocessed flowers of *Apocynum cannabinum* L. the pollen-transport adhesive has a gel-like consistency in flowers at anthesis and is hardened only in older, partially desiccated flowers. Which state of the pollen-transport adhesive functions in pollination and whether it functions as a translator (i.e., each mass of adhesive removed as a single unit with a load of pollen by the pollinator) remains unknown and can only be determined with pollination studies. To date, none of the pollination studies conducted on *Apocynum* have addressed this question (Waddington, 1976; Johnson et al., 1998; Lipow & Wyatt, 1999), and none have been conducted on *Forsteronia*. The restriction or not of adhesive secretion to five discontinuous alternistaminal zones on the style-head could be coded as a character, but we do not have sufficient information on its occurrence in Apocynoideae to score it at this time. Translators are coded as present only in Periplocoideae, Secamonoideae, and Asclepiadoideae, where removal and transport of translators by pollinators has been documented (Ollerton & Liede, 1997). There are many structural and developmental similarities among translators of Periplocoideae, Secamonoideae, and Asclepiadoideae (Safwat, 1962; Kunze, 1993; Endress, 2001). Omlor (1996) suggested that translators of Periplocoideae are not homologous with those of Secamonoideae because of differences in their histochemistry during development. It is not necessary, however, for structures to be identical at every level of organization in order to be treated as potential homologs in a cladistic analysis (Freudenstein et al., 2003). Differences in histochemistry among translators and other style-head secretions could be coded as additional characters, but we do not have sufficient data to do so at this time.

8. **Nectar secretion**: 0 = absent or not evident; 1 = on lobes around ovary, these sometimes fused into an annulus; 2 = in the alternistaminal sectors of the staminal feet or the fused basal tube. Most Apocynoideae have prominent lobed or annular nectaries surrounding the ovary. Exceptions are *Wrightia*, *Pleioceras*, *Stephanostema*, *Nerium*, *Adenium*, *Isonema*, *Alafia*, *Strophanthus*, *Farquharia*, and *Holarrhena*; these are coded as absent or not evident because we cannot rule out nectar secretion from some other structures at this point. In the Periplocoideae, nectar is secreted in five alternistaminal sectors on the margins of the staminal feet (Venter & Verhoeven, 2001). In Secamonoideae and Asclepiadoideae, it is secreted in the alternistaminal sectors of the fused basal tube (Omlor, 1996; Endress & Bruyns, 2000). Because we have chosen to interpret the staminal feet and the fused basal tube as outgrowths of the stamen–corolla tube (char. 4), we here code the presence of nectaries in alternistaminal sectors of the stamen–corolla tube as homologous for Secamonoideae, Periplocoideae, and Asclepiadoideae.

9. **Micropylar coma on seed**: 0 = absent; 1 = present. A micropylar coma is present in most taxa in the APSA clade, but absent in *Wrightia*, *Pleioceras*, *Stephanostema*, *Kibatalia* G. Don., *Funtumia* Stapf, and *Malouetia* A. DC. The existence of taxa with a micropylar coma only, the most frequent state in the APSA clade, with a chalazal coma only, with both micropylar and chalazal comas (*Adenium*, *Strophanthus*, *Isonema*, and *Farquharia*), and with no coma (*Malouetia*), indicates that presence or absence of a coma at one end of the seed is independent of presence or absence at

the other end of the seed and should be treated as two independent characters.

10. **Chalazal coma on seed**: 0 = absent; 1 = present. Chalazal comas are rare in the APSA clade, present only in *Wrightia*, *Pleioceras*, *Stephanostema*, *Kibatalia*, *Funtumia*, *Adenium*, *Strophanthus*, *Isonema*, and *Farquharia* (Van der Ploeg, 1983; Sennblad et al., 1998). All genera with chalazal comas have been classified in the tribe Wrightieae and/or Malouetieae in recent classifications.

11. **Pollen apertures**: 0 = absent; 1 = present. Most Rauvolfioideae and Apocynoideae have aperturate pollen with colporate or porate apertures, respectively. An exception is *Condylocarpon* Desf. (Rauvolfioideae, Alyxieae) with inaperturate pollen (Van der Ham et al., 2001; Endress et al., 2007). Periplocoideae have porate pollen; Secamonoideae and Asclepiadoideae have inaperturate pollen (Verhoeven & Venter, 2001; Verhoeven et al., 2003).

12. **Pollen unit at maturity**: 0 = monads; 1 = tetrads. Pollen in most Apocynaceae is dispersed as monads; tetrads are present in *Condylocarpon* (Van der Ham et al., 2001), *Apocynum* (Nilsson et al., 1993), Periplocoideae, Secamonoideae, and Asclepiadoideae. Pollinia of Asclepiadoideae other than *Fockea* Endl. have been described as composed of single pollen grains (Verhoeven et al., 2003); however, there is no free microspore stage in the development of these pollinia, rather the linear microspore tetrads are obscured as the grains enlarge and displace each other (Safwat, 1962; Dan Dicko-Zafimahova, 1980).

13. **If tetrads, dispersed as**: 0 = solitary tetrads; 1 = tetrads coherent into pollinia. A pollinium is the coherent mass of the entire pollen content of single sporangium. All Secamonoideae and Asclepiadoideae have pollen aggregated into pollinia, as do seven genera of Periplocoideae (Verhoeven & Venter, 1998; Ionta & Judd, 2007); two of these, *Gymnanthera* R. Br. and *Finlaysonia* Wall., are sampled here. The presence of pollinia is here treated as a potential homology shared by these genera with Secamonoideae and Asclepiadoideae. Taxa that have pollen in monads (char. 12, 0) are scored as inapplicable for this character.

14. **Pollinial wall**: 0 = absent; 1 = present. A sporopollenin wall enclosing the entire pollinium is present in all Asclepiadoideae studied to date except *Fockea* (Verhoeven et al., 2003). Pollinia of Secamonoideae and Periplocoideae also lack a pollinial wall (Verhoeven & Venter, 2001). This character is scored only for taxa that have pollinia (char. 13, 1).

15. **Tetrads**: 0 = outer and inner walls not differentiated; 1 = outer and inner walls differentiated. In tetrads of *Condylocarpon* (Rauvolfioideae), Periplocoideae, Secamonoideae, and *Fockea* (Asclepiadoideae) the inner walls are differentiated from the outer walls by the presence of intine bridges (Van der Ham et al., 2001; Verhoeven & Venter, 2001). No such differentiation of outer and inner tetrad walls is present in *Apocynum* (Nilsson et al., 1993) and Asclepiadoideae other than *Fockea* (Verhoeven & Venter, 2001). This character is scored as inapplicable for taxa with pollen dispersed as monads (char. 12. 0).

16. **Number of sporangia**: 0 = 4; 1 = 2. Reduction of sporangia from four to two is apomorphic for Asclepiadoideae (Kunze, 1996). The presence of four sporangia and four pollinia is plesiomorphic in the Secamonoideae and has been used to interpret the subfamily as transitional between Periplocoideae and Asclepiadoideae (Safwat, 1962).

Appendix 3. Matrix of 16 morphological characters, arranged alphabetically by genus and species. An asterisk (*) indicates polymorphic for all states; a question mark (?), unknown; a dash (-), inapplicable.

Species	1 6 11 16
Adenium obesum	0010100011110---0
Aganosma cymosa	1010100111010---0
Aganosma marginata	1010100111010---0
Aganosma schlechteriana	1010100111010---0
Aganosma wallichii	1010100111010---0
Alafia barteri	1010100010010---0
Alafia thouarsii	1010100010010---0
Allamanda schottii	00000-010010---0
Allomarkgrafia brenesiana	1110110110010---0
Allotoonia agglutinata	1010100111010---0
Allotoonia woodsoniana	1010100111010---0
Alyxia grandis	00000-000010---0
Alyxia oblongata	00000-000010---0
Alyxia reinwardtii	10000-000010---0
Alyxia spicata	10000-000010---0
Amalocalyx microlobus	1010100111010---0
Angadenia berteroi	*0101001110100---0
Angadenia sagraei	1010100111010---0
Anodendron oblongifolium	1010100111010---0
Anodendron paniculatum	1010100111010---0
Apocynum androsaemifolium	0010100110110-00

Appendix 3. Continued.

Species	1 6 11 16 | | | |
Apocynum cannabinum	0010100110110-00
Artia balansae	101010011010---0
Asclepias syriaca	0111111210011101
Baissea multiflora	111010011010---0
Beaumontia grandiflora	101010011010---0
Carissa spinarum	00000-000010---0
Cerbera manghas	00000-000010---0
Cerbera odollam	00000-000010---0
Chilocarpus costatus	10000-000010---0
Chilocarpus rostratus	10000-000010---0
Chonemorpha fragrans	101010011010---0
Cleghornia malaccensis	101010011010---0
Condylocarpon isthmicum	10000-0000010-10
Cryptolepis sinensis	1010011210110-10
Cycladenia humilis	001010011010---0
Echites turriger	101010011010---0
Echites umbellatus	101010011010---0
Elytropus chilensis	101010011010---0
Epigynum auritum	10101?011010---0
Epigynum cochinchinense	10101?011010---0
Epigynum griffithianum	10101?011010---0
Epigynum ridleyi	10101?011010---0
Farquharia elliptica	111010001110---0
Fernaldia pandurata	101010011010---0
Finlaysonia insularum	1010011210111010
Fockea edulis	1111111210011011
Forsteronia acouci	11101*011010---0
Forsteronia corymbosa	10101?011010---0
Forsteronia guyanensis	11101*011010---0
Forsteronia velloziana	11101*011010---0
Funtumia africana	001010010110---0
Funtumia elastica	001010010110---0
Galactophora schomburgkiana	*01010011010---0
Gymnanthera oblonga	1010011210111010
Heterostemma piperifolium	1111111210011101
Himatanthus bracteatus	00000-000010---0
Holarrhena curtisii	001000001010---0
Holarrhena pubescens	001000001010---0
Ichnocarpus frutescens	10101?011010---0
Ichnocarpus polyanthus	101010011010---0
Ichnocarpus rhombifolius	101010011010---0
Ichnocarpus serpyllifolius	101010011010---0
Isonema smeathmannii	111010001110---0
Kibatalia macrophylla	001010010110---0
Laubertia contorta	101010011010---0
Malouetia bequaertiana	001010010010---0
Malouetia tamaquarina	001010010010---0
Mandevilla boliviensis	111011011010---0
Mandevilla longiflora	011011011010---0
Marsdenia glabra	1111111210011101
Mascarenhasia arborescens	001010011010---0
Mascarenhasia lisianthiflora	001010011010---0
Mesechites trifidus	111011011010---0
Motandra guineensis	111010011010---0
Neobracea bahamensis	001010011010---0

Appendix 3. Continued.

Species	1 6 11 16 | | | |
Neobracea ekmannii	001010011010---0
Neobracea valenzuelana	001010011010---0
Nerium oleander	011010001010---0
Odontadenia lutea	101010011010---0
Odontadenia perrottetti	101010011010---0
Odontadenia puncticulosa	101010011010---0
Oncinotis tenuiloba	111010011010---0
Pachypodium baronii	001010011010---0
Pachypodium lamerei	001010011010---0
Papuechites aambe	101010011010---0
Parameria laevigata	10001?011010---0
Parsonsia crebriflora	102010011010---0
Parsonsia eucalyptophylla	102010011010---0
Parsonsia ferruginea	102010011010---0
Parsonsia flexuosa	102010011010---0
Parsonsia lata	101010011010---0
Parsonsia longiflora	102010011010---0
Parsonsia oligantha	101010011010---0
Peltastes isthmicus	101010011010---0
Peltastes peltatus	101010011010---0
Pentalinon luteum	101010011010---0
Petopentia natalensis	101001121011O-10
Phyllanthera grayi	10110112101l0-10
Plectaneia thouarsii	10000-000010---0
Pleioceras barteri	*00010000110---0
Plumeria cubensis	00000-000010---0
Pottsia laxiflora	101010011010---0
Prestonia coalita	101010011010---0
Prestonia lagoensis	101010011010---0
Prestonia mexicana	101010011010---0
Prestonia portobellensis	101010011010---0
Prestonia quinquangularis	101010011010---0
Prestonia riedelii	101010011010---0
Prestonia robusta	101010011010---0
Prestonia tomentosa	101010011010---0
Pteralyxia kauaiensis	00000-000010---0
Raphionacme flanaganii	101001121011O-10
Rhabdadenia biflora	101010011010---0
Rhabdadenia macrostoma	101010011010---0
Rhabdadenia pohlii	*01010011010---0
Rhodocalyx rotundifolius	001010011010---0
Salpinctes kalmiifolius	*01010011010---0
Secamone elliptica	101111121001l010
Secondatia densiflora	*01010011010---0
Sindechites chinensis	10101?011010---0
Sindechites henryi	10101?011010---0
Spirolobium cambodianum	001010011010---0
Stephanostema stenocarpum	000010000110---0
Stipecoma peltigera	101010011010---0
Strophanthus boivinii	001010001110---0
Strophanthus caudatus	101010001110---0
Strophanthus preussii	*01010001110---0
Telosma cordata	111111121001l101
Temnadenia odorifera	101010011010---0
Temnadenia violacea	101010011010---0

Appendix 3. Continued.

Species	1 6 11 16 | | | |
Thevetic peruviana	00000-010010---0
Toxocarpus peruviana	1101111210011010
Trachelospermum asiaticum	101010011010---0
Trachelospermum axillare	101010011010---0
Trachelospermum difforme	101010011010---0
Trachelospermum jasminoides	101010011010---0
Urceola lucida	102010011010---0
Urceola micrantha	101010011010---0
Urceola minutiflora	102010011010---0
Urceola rosea	101010011010---0
Vallaris solanacea	101010011010---0
Vincetoxicum rossicum	1111111210011101
Wrightia arborea	000010000110---0
Wrightia coccinea	000010000110---0
Wrightia dubia	000010000110---0
Wrightia lanceolata	000010000110---0
Wrightia religiosa	000010000110---0
Wrightia sirikitiae	000010000110---0
Zygostelma benthamii	1010011210110-10

Appendix 4. Resurrected genera. Taxonomic changes requiring new combinations will be published separately.

Amphineurion (A. DC.) Pichon, Bull. Soc. Bot. Fr. 95: 215. 1948. TYPE: *Amphineurion marginatum* (A. DC.) D. J. Middleton. 1 species.

Microchites Miq., Fl. Ind. Bat. 2: 456. 1856. TYPE: *Microchites polyantha* (Blume) Miq. 10 species.

Rhodocalyx Müll. Arg., in Martius, Fl. Bras. 6(1): 172. 1860. TYPE: *Rhodocalyx rotundifolius* Müll. Arg. 1 species.

Salpinctes Woodson, in Gleason, Bull. Torrey Bot. Club. 58: 453. 1931. TYPE: *Salpinctes kalmiifolius* Woodson. 2 species.

Thyrsanthella (Baill.) Pichon, Bull. Mus. Hist. Nat. Paris. Sér. 2. 20: 192. 1948. TYPE: *Thyrsanthella difforme* (Walter) Pichon. 1 species.

PHYLOGENETIC RELATIONSHIPS IN PERIPLOCOIDEAE (APOCYNACEAE S.L.) AND INSIGHTS INTO THE ORIGIN OF POLLINIA[1]

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ABSTRACT

Phylogenetic relationships within Periplocoideae (Apocynaceae s.l.) were investigated using sequence data from nuclear ribosomal DNA and two plastid regions: *trnD-trnT* and *trnT-trnF*. We included 43 ingroup taxa, representing all but three of 32 currently recognized genera. In the ITS and total evidence analyses, *Phyllanthera grayi* (P. I. Forst.) Venter is supported as sister to the rest of the subfamily. We identified several well-supported clades, and three of six genera represented here by more than one species (*Cryptolepis* R. Br., *Pentopetia* Decne., and *Periploca* L.) are supported as monophyletic. Pollinial species appear in four distinct, well-defined clades, suggesting either three or four independent origins of pollinia within the subfamily. It is most parsimonious, therefore, to view pollinia in Periplocoideae as non-homologous with those of Secamonoideae and Asclepiadoideae, a hypothesis that is supported by their structural differences.

Key words: Apocynaceae, character evolution, molecular phylogeny, Periplocoideae, pollinium, tetrad, translator.

Periplocoideae comprise ca. 190 species in 32 genera found throughout the Old World tropics and subtropics and centered in Africa, Asia, and Madagascar; some species occur in temperate regions and a few are found in Australia and Europe. The majority are woody climbers occupying tropical and subtropical forests; other forms include erect or straggling shrubs from semi-arid and desert regions, suffrutescent forest climbers, herbaceous geophytes from open habitats, and epiphytes in tropical forests (Venter, 1997; Venter & Verhoeven, 1997, 2001). The generally small (most under 10 mm long) yet extremely complex flowers of Periplocoideae exhibit the high degree of synorganization between androecium and gynoecium typical for advanced members of Apocynaceae s.l. Floral form varies among as well as within genera. This is evidenced by a wide range of corolla shapes, an elaborate diversification of corolline coronas, varying degrees of fusion between coronas and stamens, and differences in the position of floral organs on the corolla tube. Venter and Verhoeven (1997, 2001) provide an excellent review of the range of vegetative and floral diversity present in Periplocoideae.

Over the years Periplocoideae have been included in Asclepiadaceae (Brown, 1810; Bentham, 1876; Schumann, 1895; Liede & Albers, 1994), considered a separate family (Schlechter, 1905; Bullock, 1956; Kunze, 1993; Omlor, 1996; Swarupanandan et al., 1996), or recognized as part of a more inclusive Apocynaceae along with other Asclepiadaceae (Safwat, 1962; Judd et al., 1994; Sennblad & Bremer, 1996; Venter & Verhoeven, 1997; Endress & Bruyns, 2000; Venter & Verhoeven, 2001). Brown (1810) included Periplocoideae, Asclepiadoideae, and Secamonoideae in Asclepiadaceae on the basis of morphological similarities, primarily the presence of translators (complex pollen carriers formed from hardened secretions of the stylar head) in these taxa. The relatively soft translators of Periplocoideae consist of a spoon-shaped receptacle onto which pollen is shed at anthesis, an adhesive disc that attaches to the pollinator, and a stipe or stalk connecting the two (Safwat, 1962; Schick, 1982; Verhoeven & Venter, 1997). In most taxa, pollen is shed as separate tetrads; seven genera have been documented as having tetrads aggregated into pollinia (Verhoeven & Venter, 1998a, b, 2001, 2002). In

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Asclepiadoideae and Secamonoideae, single pollen grains or tetrads are aggregated into pollinia that are firmly attached to hardened, clip-like translators before anthesis. These differences in pollen and translator morphology led Schlechter (1905) to recognize Periplocaceae as a separate family. Today, most researchers agree that Periplocoideae, Asclepiadoideae, and Secamonoideae belong in an expanded Apocynaceae s.l. A number of phylogenetic analyses support this grouping, as well as the monophyly of Periplocoideae (Sennblad & Bremer, 1996; Civeyrel et al., 1998; Potgieter & Albert, 2001; Lahaye et al., 2005), and recent studies show that Periplocoideae are likely nested within the paraphyletic grade Apocynoideae (Sennblad & Bremer, 1996; Potgieter & Albert, 2001).

The number of accepted genera in Periplocoideae has changed considerably through the years and is still in flux. Given the small size of the subfamily, a disproportionately high number of monotypic and ditypic genera have been named. This may be due to a variety of factors: the group is extremely diverse, closely related species may differ greatly in morphology, many taxa (particularly in Asia) have not been extensively collected and are poorly known (Venter & Verhoeven, 1997; Meve & Liede, 2004), and the small yet highly complex flowers are difficult to interpret from herbarium material. A total of 86 genera have been named in Periplocoideae (Venter & Verhoeven, 1997). Venter and Verhoeven proposed a tribal classification of the subfamily in 1997, which included 44 genera in three tribes. Since then, a number of smaller genera were placed in synonymy by Klackenberg (1997, 1998, 1999) and Venter and Verhoeven (2001), reducing the number of genera to 31. Today approximately half of the species belong to one of three large genera: *Raphionacme* Harv. (37 species), *Cryptolepis* R. Br. (27), and *Pentopetia* Decne. (21). The remaining genera contain from one to 14 species; nine are monotypic and eight ditypic. Here we follow the generic circumscription of Venter and Verhoeven (2001) with one exception: in accordance with Meve and Liede (2004), we recognize the African *Petopentia natalensis* (Schltr.) Bullock as separate from the Malagasy endemic genus *Ischnolepis* Jum. & H. Perrier.

In his publications, N. E. Brown (1902, 1904) used variations in the length of the upper corolla tube to delimit genera within the subfamily. Venter and Verhoeven (1997) also emphasized floral features in a tribal classification of Periplocoideae based on overall corolla form, exposure of the gynostegium from the corolla, and the position of coronas and stamens in the corolla tube. A subsequent phylogenetic analysis of the subfamily by Venter and Verhoeven (2001) based on morphological data did not support the tribes proposed in their earlier classification, and they noted that, within Periplocoideae, floral features such as the length of the corolla tube were more variable than previously thought.

Of the five subfamilies that form Apocynaceae s.l., Periplocoideae are unique in having taxa both with and without pollinia. Pollinia have been documented in seven genera: five Asian (*Decalepis* Wight & Arn., *Finlaysonia* Wall., *Gymnanthera* R. Br., *Hemidesmus* R. Br., and *Streptocaulon* Wight & Arn.) and two African (*Epistemma* D. V. Field & J. B. Hall and *Schlechterella* K. Schum.). Pollinia in Periplocoideae differ markedly from those found in other Apocynaceae: they are shed onto the translator at anthesis (in Secamonoideae and Asclepiadoideae the pollinia are firmly attached to hardened corpuscles before being removed from the anther sacs by pollinators), they lack the pollinium walls (ectexine) of Asclepiadoideae, and the tetrads that form a pollinium are only loosely agglutinated. Venter and Verhoeven (1997) hypothesized that pollinia in Periplocoideae evolved polyphyletically. Later (Venter & Verhoeven, 2001), they predicted an independent origin of pollinia in Asian and African Periplocoideae due to differences in the pollinium structure of *Schlechterella* as compared to that of other pollinial taxa that had been documented by them (Verhoeven & Venter, 1998b). Fishbein (2001: 609) explored the origins and diversification of pollinia in the former Asclepiadaceae, concluding that "pollinia of Periplocoideae are not reconstructed as homologous with those of other asclepiads. . . it appears that pollinia may have evolved independently on several occasions in Periplocoideae, based on the distribution of pollinia among putatively isolated genera in each of the three recognized tribes."

Our primary goal in undertaking this study was to elucidate phylogenetic relationships within Periplocoideae. To that end, we utilized the ITS regions of nuclear ribosomal DNA and two plastid regions: *trnT-trnF* (the *trnT-trnL* intergenic spacer, the *trnL-trnF* spacer, and the *trnL* intron) and the *trnD-trnT* spacer. The *trnT-trnF* region (in part or in whole) has been used successfully to assess relationships among genera throughout Apocynaceae s. str. (Liede, 2001; Meve & Liede, 2001, 2002, 2004; Lahaye et al., 2005). The resulting hypotheses of phylogenetic relationships are compared with the tribes of Venter and Verhoeven (1997) and the results of former phylogenetic analyses.

Material and Methods

Taxon Sampling

Forty-five taxa representing 29 of 32 currently recognized Periplocoideae genera (sensu Venter &

Verhoeven, 2001, plus *Petopentia* Bullock) were included in this study, along with two outgroup taxa (Table 1). Outgroup choice of *Forsteronia leptocarpa* A. DC. and *Rhabdadenia biflora* Müll. Arg. was based on recent molecular studies nesting Periplocoideae within Apocynoideae (Sennblad & Bremer, 1996; Potgieter & Albert, 2001). We were unable to obtain sufficient material for DNA extraction of three genera: *Sarcorrhiza* Bullock, *Telectadium* Baill., and *Maclaudia* Venter & R. L. Verh.

Leaf tissue was obtained from live plants grown in the University of Florida Department of Botany greenhouse, from field-collected specimens dried in silica (generously donated by a number of researchers, see acknowledgments) and from herbarium material. Genomic DNA was isolated using a modification of the CTAB extraction protocol outlined by Doyle and Doyle (1987). Extracted DNA was purified using Qiagen QIAQuick columns (Qiagen, Santa Clarita, California, U.S.A.) using the manufacturer's protocol.

PCR AMPLIFICATION AND DNA SEQUENCING

Ribosomal DNA was amplified in 50-μl reactions with primers 17SE and 26SE of Sun et al. (1994), using a touchdown thermal cycling program with an initial annealing temperature of 76°C, decreasing 1°C per cycle for 15 cycles, followed by 15 cycles at 61°C. With difficult samples (e.g., herbarium specimens), ITS-1 and ITS-2 were amplified separately using the primers and protocol of Blattner (1999).

The *trnD-trnT* intergenic spacer region was amplified and sequenced using the primers and protocol of Demesure et al. (1995). The *trnT-trnF* chloroplast region spans *trnT-trnL*, *trnL-trnF*, and the *trnL* intron. We amplified and sequenced this region using the primers and protocol of Taberlet et al. (1991), with the exception of their trnTR primer, which we substituted with trnT2F of Shaw et al. (2005). Attempts to amplify chloroplast regions from genomic DNA extracted from herbarium specimens often proved unsuccessful, resulting in fewer exemplars in the chloroplast region matrices.

PCR products were purified using Qiagen QIAQuick columns (Qiagen) using the manufacturer's protocol. Sequencing reactions were performed using Big Dye V3.1 chemistry. Primers used for cycle sequencing were the same as those used in PCR amplification. All sequencing was done on Applied Biosystems ABI 377 Sequencers (Applied Biosystems, Foster City, California, U.S.A.) at the Interdisciplinary Center for Biotechnology Research, University of Florida. In addition to the sequences generated for this study, we included sequences previously published by Meve and Liede (2004).

PHYLOGENETIC ANALYSES

Sequences were manually edited and assembled using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.), and consensus sequences were visually aligned using SeAl 2.0 (Rambaut, 1996). Regions of ambiguous alignment were identified and excluded from analyses. Parsimony analyses were conducted using PAUP* 4.0b10 (Swofford, 1999). For each heuristic search all characters were equally weighted, character state transitions were treated as unordered, and gaps were treated as missing data. Each matrix underwent 1000 random addition replicates, saving 10 trees per replicate, with tree bisection-reconnection (TBR) branch swapping, and with the MULTREES option in effect. The shortest trees retained in memory were then swapped to completion. Relative support for clades was evaluated using 1000 bootstrap (BS) replicates (Felsenstein, 1985), with 10 random addition replicates and subtree pruning and regrafting (SPR) branch swapping.

Congruence of separate data sets was assessed using a conditional combination approach (Huelsenbeck et al., 1996), as described by Soltis et al. (2001). First, each data set was analyzed separately. We then compared the resultant topologies and searched for instances of strongly supported contradictory clades. Following the approach of Mason-Gamer and Kellogg (1996), we established a bootstrap value cutoff of 70% for assessing strong conflict between data sets. The chloroplast is inherited as a single unit and is not subject to recombination; therefore, we treated the plastid data sets as a single linkage partition. Congruence of nuclear and plastid data was further assessed using a partition homogeneity test, as implemented in PAUP* 4.0b10.

Analyses of the following data sets were performed: (1) ITS alone, comprising 43 ingroup taxa in 29 genera and two outgroup taxa; (2) *trnT-trnF*, with 27 ingroup taxa in 18 genera and two outgroup taxa; (3) the *trnD-trnT* region, with 21 ingroup taxa in 17 genera and one outgroup taxon; (4) a combined chloroplast data set containing 28 ingroup taxa in 19 genera and two outgroup taxa; and (5) a combined total evidence data set of 45 taxa (43 ingroup taxa in 29 genera and two outgroup taxa). Chloroplast sequences are missing for several taxa, and as a result the data sets for analyses (4) and (5) are incomplete; missing sequences are indicated by dashes in Table 1.

RESULTS

ITS

The aligned ITS matrix was 774 characters long; 265 of 399 variable characters were parsimony

informative. Parsimony analysis resulted in 93 most parsimonious trees (MPT) of 1223 steps (consistency index [CI] = 0.474; retention index [RI] = 0.609; rescaled consistency index [RC] = 0.289); the strict consensus of these trees is shown in Figure 1. *Phyllanthera grayi* (P. I. Forst.) Venter, an Asian taxon, is supported as sister to the rest of the subfamily (BS 99). A large, well-supported clade (BS 90; herein referred to as the grooved translator clade) contains 23 Asian and African taxa, including the eight pollinial taxa included in this analysis. Within this clade, there is moderate support (BS 72) for an Asian clade of seven taxa (the Asian pollinial clade), the majority of which (five of seven) bear pollinia. Within this Asian pollinial clade, the monophyly of *Finlaysonia*, represented in this study by *F. insularum* (King & Gamble) Venter and *F. lanuginosa* (Ridl.) Venter, is not supported. *Finlaysonia lanuginosa* is sister to *Streptocaulon juventas* (Lour.) Merr. (BS 71), while *F. insularum* forms a clade with *Atherandra acuminata* Decne.–*Gymnanthera oblonga* (Burm. f.) P. S. Green–*Zygostelma benthamii* Baill. (BS 89). Sister to this Asian pollinial clade (BS 95) is a clade containing two African taxa: *Buckollia volubilis* (Schltr.) Venter & R. L. Verh. (tetrads) and *Epistemma rupestre* H. Huber (pollinia-bearing). *Raphionacme* is paraphyletic, with all but one species forming a clade (BS 100); the exception, *Raphionacme lobulata* Venter & R. L. Verh., is sister to the genus *Stomatostemma* N. E. Br. (BS 59). Four of five *Periploca* L. species included in this analysis form a clade (BS 100); the exception, *P. nigrescens* Afzel., is sister to *Omphalogonus calophyllus* Baill. (BS 100). *Cryptolepis* and *Pentopetia*, relatively large genera represented here by five of 27 species and two of 21 species, respectively, are both supported as monophyletic (BS 99, BS 97).

PLASTID REGIONS

The aligned *trnT-trnF* matrix was 1757 characters long after removal of sites where alignment was ambiguous; 57 of 231 variable characters were parsimony informative. Parsimony analysis yielded 9890 shortest trees of 262 steps (CI = 0.931; RI = 0.841; RC = 0.783). The aligned *trnD-trnT* matrix was 1292 characters long after removal of sites where alignment was ambiguous; 20 of 194 variable characters were parsimony informative. Parsimony analysis yielded 9010 shortest trees of 212 steps (CI = 0.948; RI = 0.667; RC = 0.632).

Visual inspection of the strict consensus trees from the separate *trnT-trnF* and *trnD-trnT* analyses revealed no strongly supported contradictory clades. Therefore, we combined the two matrices into a single plastid data set. Sampling was uneven due to eight missing *trnD-trnT* sequences and one missing *trnT-trnF* sequence, representing 13.2% of the data. Parsimony analysis yielded 9310 MPT of 475 steps (CI = 0.939; RI = 0.801; RC = 0.752); the strict consensus of those trees is presented in Figure 2. Combining the plastid data sets resulted in greater support for the clades present in the separate plastid analyses, with additional groupings not present in the ITS or separate plastid analyses. A clade containing *Camptocarpus mauritianus* (Lam.) Decne., *Cryptostegia madagascariensis* Bojer, *Ischnolepis graminifolia* (Constantin & Gallaud) Klack., *Pentopetia grevei* (Baill.) Venter, and *P. longipetala* Klack., representing four of five Malagasy genera (*Baroniella* Costantin & Gallaud is absent from this data set) is supported (BS 82). Four genera are monophyletic: *Cryptolepis* (BS 79), *Pentopetia* (BS 68), *Periploca* (BS 100; *P. nigrescens* absent from plastid data set), and *Raphionacme* (BS 97; *R. lobulata* absent from plastid data set). *Ectadium latifolium* N. E. Br. is weakly supported as sister to *Periploca* (64% BS); this relationship was not supported by the ITS analysis. *Schlechterella abyssinica* (Chiov.) Venter & R. L. Verh. is sister to *Raphionacme* (BS 72). The Asian pollinial clade identified in the ITS analysis is represented in the combined plastid analysis by four taxa, which again form a clade (BS 81). Within this clade, *Finlaysonia* is polyphyletic, in agreement with the results of the ITS analysis.

TOTAL EVIDENCE

Visual inspection of the separate analyses of the nuclear and plastid data sets revealed no strongly supported contradictory clades, and a partition homogeneity test showed that the plastid and ITS data sets are concordant ($P = 0.28$); therefore, we combined the data sets. Sampling was uneven due to 24 missing *trnD-trnT* sequences and 17 missing *trnT-trnF* sequences, representing 34.6% of the data. Parsimony analysis of the total evidence data set resulted in 142 MPT of 1706 steps (CI = 0.601; RI = 0.620; RC = 0.373); one of the 142 shortest trees is presented in Figure 3. This analysis yielded a strict consensus tree similar in topology to the ITS consensus tree, with better resolution in portions of the tree and better support for most nodes. As in the ITS analysis, *Phyllanthera grayi* is supported as sister to the rest of the subfamily, a relationship not seen in the plastid analyses. The Asian pollinial clade (BS 71), the grooved translator clade (BS 94), the *Cryptolepis–Omphalogonus calophyllus–Periploca nigrescens* clade (BS 97), and the *Periploca laevigata* Ait. through *P.*

Table 1. Voucher information for plant material used in this study. Sequences previously published by Meve and Liede (2004) are indicated with an asterisk. Dashes indicate missing sequences. Two GenBank numbers are given for the *trnT-F* region of *Sacleuxia newii*; the first number represents *trnL-F* and the second (last two digits given) represents *trnT-L*.

Taxon	Origin	Voucher	GenBank accession numbers		
			trnT-F	*trnD-T*	ITS
OUTGROUP					
Forsteronia leptocarpa A. DC.	Brazil, Ubatuba	*Simões & Singer 1037* (UEC)	DQ916868	—	DQ916831
Rhabdadenia biflora Müll. Arg.	U.S.A., Florida	*Zona 616* (FTG)	DQ916869	DQ916903	DQ916832
PERIPLOCOIDEAE					
Atherandra acuminata Decne.	Thailand, Surat	*Put. 4139* (P)	—	—	DQ916833
Baroniella camptocarpoides Costantin & Gallaud	Madagascar, Toliara	*Phillipson et al. 3956* (MO)	—	—	DQ916834
Baseonema gregorii Schltr. & Rendle	Tanzania	*Cox & Abdallah 2028* (K)	—	—	DQ916835
Batesanthus purpureus N. E. Br.	Zaire	*Hart 620* (MO)	—	—	DQ916836
Buckollia volubilis (Schltr.) Venter & R. L. Verh.	Ethiopia, Sidamo	*Hangen 2041* (K)	—	—	DQ916837
Camptocarpus mauritianus (Lam.) Decne.	Madagascar, Toliara	*Petignat s.n.* (UBT)	*	DQ916887	*
Cryptolepis buchananii Roem. & Schult.	Pakistan, Islamabad	*Sultan s.n.* (UBT)	DQ916870	DQ916888	DQ916838
Cryptolepis capensis Schltr.	South Africa	*Abbott 7761* (Z)	DQ916871	DQ916889	DQ916839
Cryptolepis delagoensis Schltr.	South Africa, KwaZulu-Natal	*Bruyns 9357* (BOL)	—	—	DQ916840
Cryptolepis hypoglauca K. Schum. ex Engl.	Tanzania, Masasi Distr.	*Bidgood et al. 2086* (K)	—	—	DQ916841
Cryptolepis oblongifolia (Meisn.) Schltr.	South Africa, Transvaal	*Bruyns 9367* (E)	DQ916872	DQ916890	DQ916842
Cryptostegia madagascariensis Bojer	U.S.A., Florida, cult.	*Ionta 118* (FLAS)	DQ916873	DQ916891	DQ916843
Cryptostegia madagascariensis Bojer	U.S.A., Florida, cult.	*Ionta 538* (FLAS)	—	—	DQ916844
Decalepis arayalpathra (J. Joseph & V. Chandras.) Venter	India, Cirzar Hills, 1989	*Rajasekharan s.n.* (K)	—	—	DQ916845
Ectadium latifolium N. E. Br.	Namibia	*Albers 2413* (UBT)	DQ916874	DQ916892	DQ916846
Epistemma rupestre H. Huber	Cameroon, Rocher Ako'okas	*deWilde 7462* (MO)	—	—	DQ916847
Finlaysonia insularum (King & Gamble) Venter	Thailand	*Middleton 1164* (A)	DQ916875	DQ916893	DQ916848
Finlaysonia lanuginosa (Ridl.) Venter	U.S.A., Cornell Univ., cult.	*Livshultz 03-41* (BH)	DQ916876	DQ916894	DQ916849
Gymnanthera oblonga (Burm. f.) P. S. Green	Australia, Northern Territory	*Forster 6133* (K)	—	—	DQ916850
Hemidesmus indicus (L.) R. Br. ex Schult.	India	*Civeyrel 1008* (TL)	DQ916877	DQ916895	DQ916851
Ischnolepis graminifolia (Costantin & Gallaud) Klack.	Madagascar, Ambinanitelo	*Röösli s.n.* (UBT)	*	—	*
Mondia whiteii (Hook. f.) Skeels	South Africa, Univ. Natal Pietermaritzberg, cult.	*ex hort. Ollerton s.n.*	DQ916878	DQ916896	DQ916852

Table 1. Continued.

Taxon	Origin	Voucher	GenBank accession numbers		
			trnT-F	*trnD-T*	ITS
Myriopteron paniculatum Griff.	s. loc.	*Shuncheng 9801135* (MO)	—	—	DQ916853
Omphalogonus calophyllus Baill.	Tanzania, Muheza District	*Mwangoka et al. 1241* (MO)	—	—	DQ916854
Pentopetia grevei (Baill.) Venter	Madagascar, Ambinanitelo	*Mangelsdorff 516* (UBT)	*	DQ916897	*
Pentopetia longipetala Klack.	Madagascar, Ambalabao	*Allorge 2422* (P)	DQ916879	—	DQ916855
Periploca calophylla (Wight) Falc.	China, Sichuan Prov.	*Liu Zheng Yu 15548* (GH)	DQ916880	—	DQ916856
Periploca graeca L.	Switzerland, Bot. Gard. Zurich, cult.	*Endress s.n.* (Z)	DQ916881	DQ916898	DQ916857
Periploca laevigata Ait.	Spain, Canary Islands	*Fernandez s.n.* (Z)	DQ916882	DQ916899	DQ916858
Periploca nigrescens Afzel.	Ghana	*Merello et al. 1354* (MO)	—	—	DQ916859
Periploca visciformis (Vatke) K. Schum.	Socotra, Wadi Ayhaft	*Mies 1444* (UBT)	*	—	*
Petopentia natalensis (Schltr.) Bullock	s. loc.	*ex hort. Kiel.* (UBT)	*	DQ916900	*
Phyllanthera grayi (P. I. Forst.) Venter	Australia	*Forster 24232* (BRI)	DQ916883	DQ916901	DQ916860
Raphionacme flanaganii Schltr.	South Africa, Pietermaritzburg	*Albers 1577* (MSUN)	*	—	*
Raphionacme galpinii Schltr.	South Africa	*Abbott s.n.* (Z)	DQ916884	DQ916902	DQ916861
Raphionacme lobulata Venter & R. L. Verh.	South Africa, Eastern Cape	*Dold 4461* (GRA)	—	—	DQ916862
Raphionacme madiensis S. Moore	Tanzania, Dodoma	*Specks 682* (UBT)	*	—	*
Sacleuxia newii (Benth.) Bullock	Tanzania, Kilosa	*Bruyns 8653* (K)	DQ916909/10	—	DQ916863
Schlechterella abyssinica (Chiov.) Venter & R. L. Verh.	Kenya, Furole	*Newton 4555* (UBT)	*	DQ916904	*
Stomatostemma monteiroae N. E. Br.	s. loc.	*IPPS 1427* (UBT)	*	DQ916905	*
Stomatostemma pendulina Venter & D. V. Field	Mozambique, Ribaue	*Bruyns 7716* (K)	—	—	DQ916864
Streptocaulon juventas (Lour.) Merr.	Laos, Sii Phan Don, Kong Distr.	*Middleton 276* (A)	—	DQ916906	DQ916865
Tacazzea apiculata Oliver	Zambia, Luapula District	*Renvoize 5760* (MO)	DQ916885	DQ916907	DQ916866
Zygostelma benthamii Baill.	Thailand, Phetchaburi	*Middleton 849* (A)	DQ916886	DQ916908	DQ916867

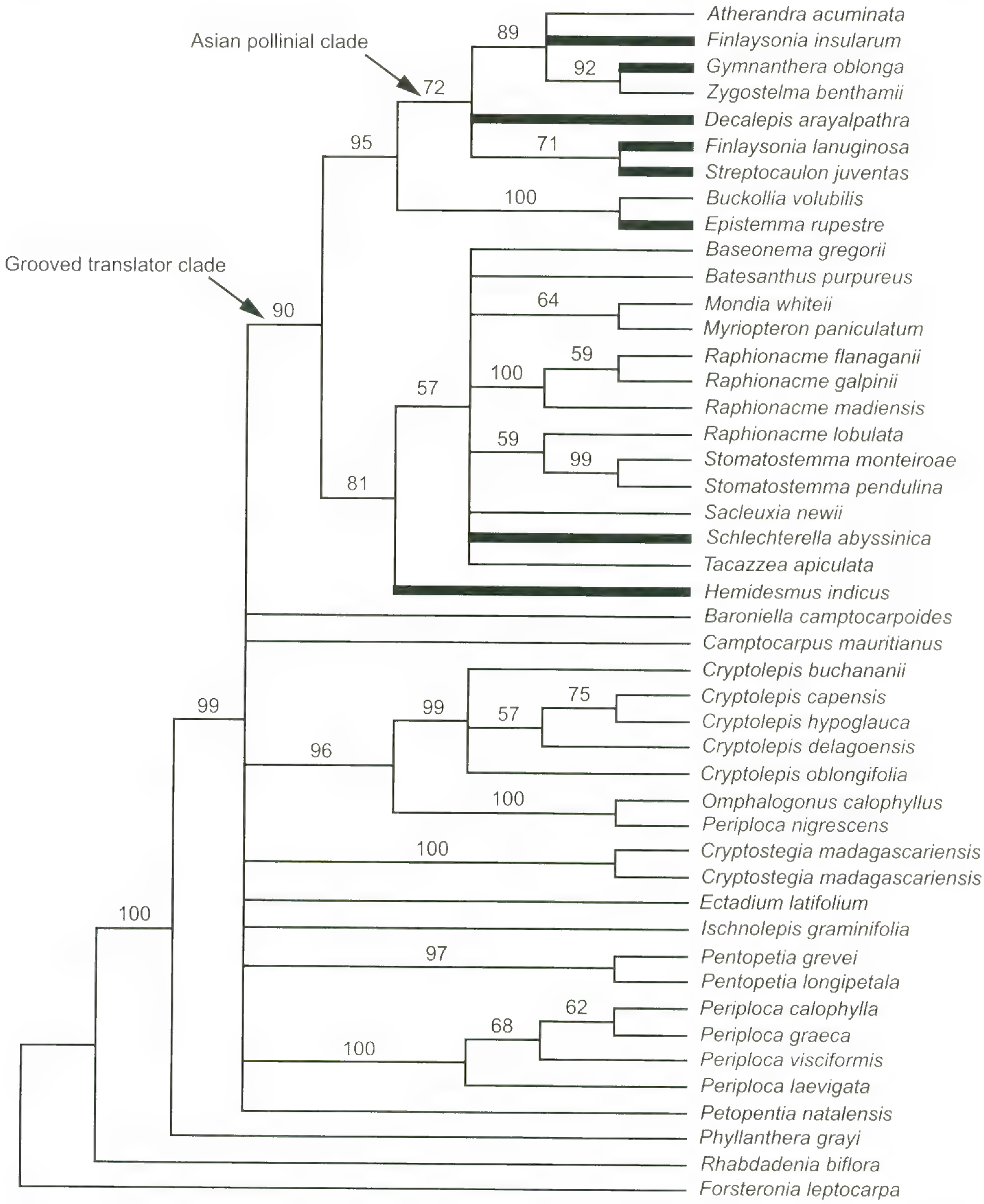

Figure 1. Strict consensus of 93 equally parsimonious trees (tree length [L] = 1223, CI = 0.474, RI = 0.609, RC = 0.289) resulting from parsimony analysis of ITS sequence data. Branches in bold indicate pollinial taxa. Numbers above the branches indicate bootstrap values.

calophylla (Wight) Falc. clade (BS 100), all present in the ITS analysis, are supported. *Finlaysonia* is polyphyletic; *F. lanuginosa* is sister to *Streptocaulon juventas* with greater support than in individual analyses (BS 75). Relationships among those taxa outside of the grooved translator clade are not well resolved. Representatives of two of five Malagasy genera (*Camptocarpus* Decne. and *Cryptostegia* R. Br.) appear together in 100% of the MPT, but there is no statistical support for this relationship.

MORPHOLOGY

Morphological assessments were made based on a combination of previously published data (Verhoeven & Venter, 1988, 1989, 1994a, b, 1997, 1998a, b,

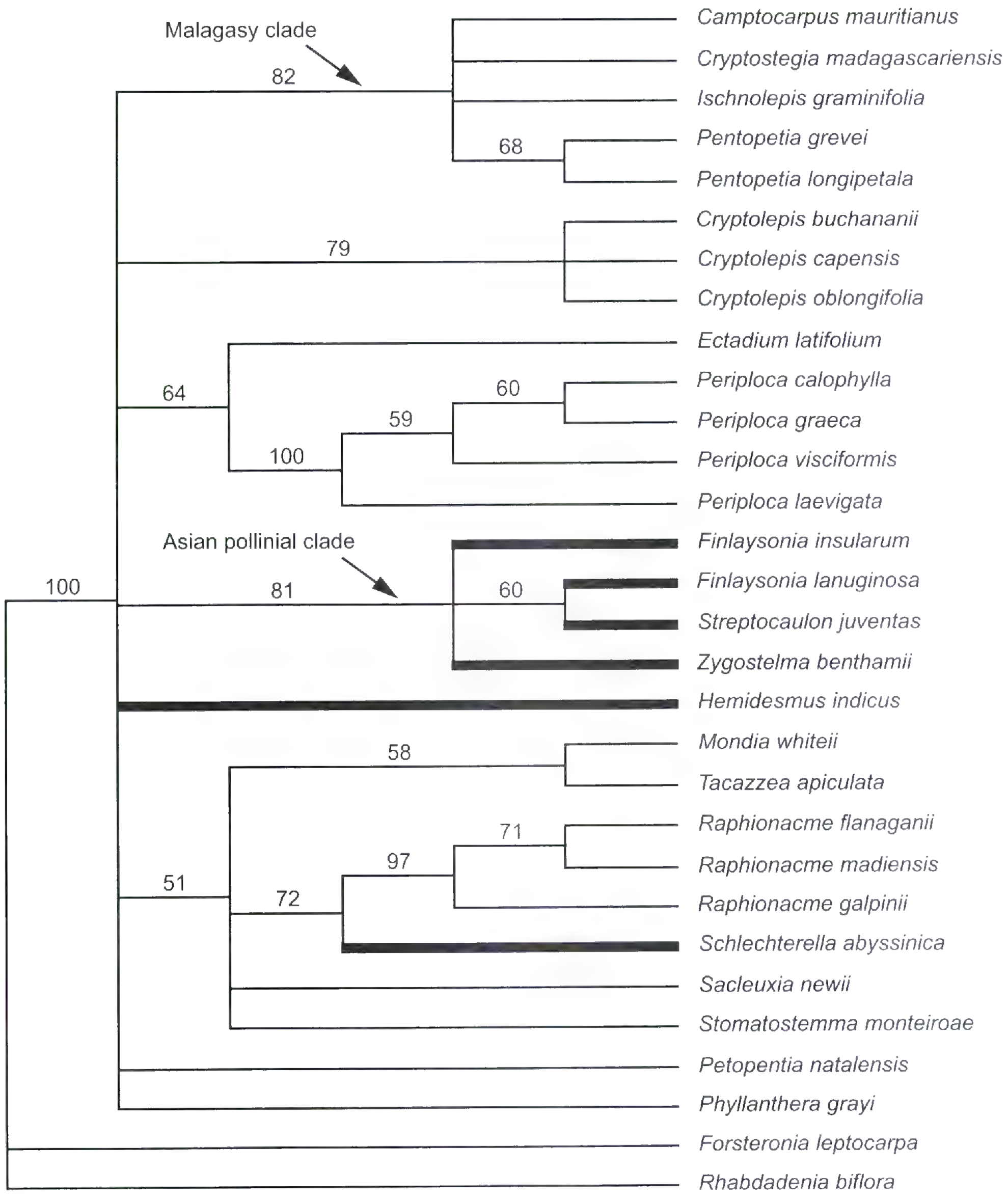

Figure 2. Strict consensus of 9310 equally parsimonious trees (L = 475, CI = 0.939, RI = 0.801, RC = 0.752) resulting from parsimony analysis of combined plastid data. Branches in bold indicate pollinial taxa. Numbers above the branches indicate bootstrap values.

2001, 2002; Verhoeven et al., 1989; Nilsson et al., 1993) and our own observations. We examined spirit-preserved flowers and/or herbarium specimens of all taxa included in this study, with a particular emphasis on pollen and pollen translators. The evolution of pollinia was analyzed by mapping the presence or absence of this character (using parsimony) onto one of the shortest trees from our total evidence analysis in MacClade 4.0 (Maddison & Maddison, 1999). Our investigation into the pollen morphology of Periplocoideae is preliminary and only summarized in this paper.

In a majority of taxa, we observed pollen tetrads (not gathered into pollinia) that were round to slightly oblong (L/W $\leq$ 1.5; Fig. 4C), with pollen grains arranged tetrahedrally, decussately, and/or rhomboidally. The exceptions were: (1) *Periploca graeca* L. (non-pollinial), in which we observed oblong (L/W = 1.5–2.0), decussate, and rhomboidal tetrads (Fig. 4E); (2) *Petopentia natalensis* (non-pollinial), with a mix of rhomboidal, linear, and T-shaped tetrad shapes (L/W = 1.5–4.0); (3) members of the Asian pollinial clade (most pollinial) with elongate (L/W = 2.0–6.0; Fig. 4G, H) T-shaped, rhomboidal, or linear tetrads;

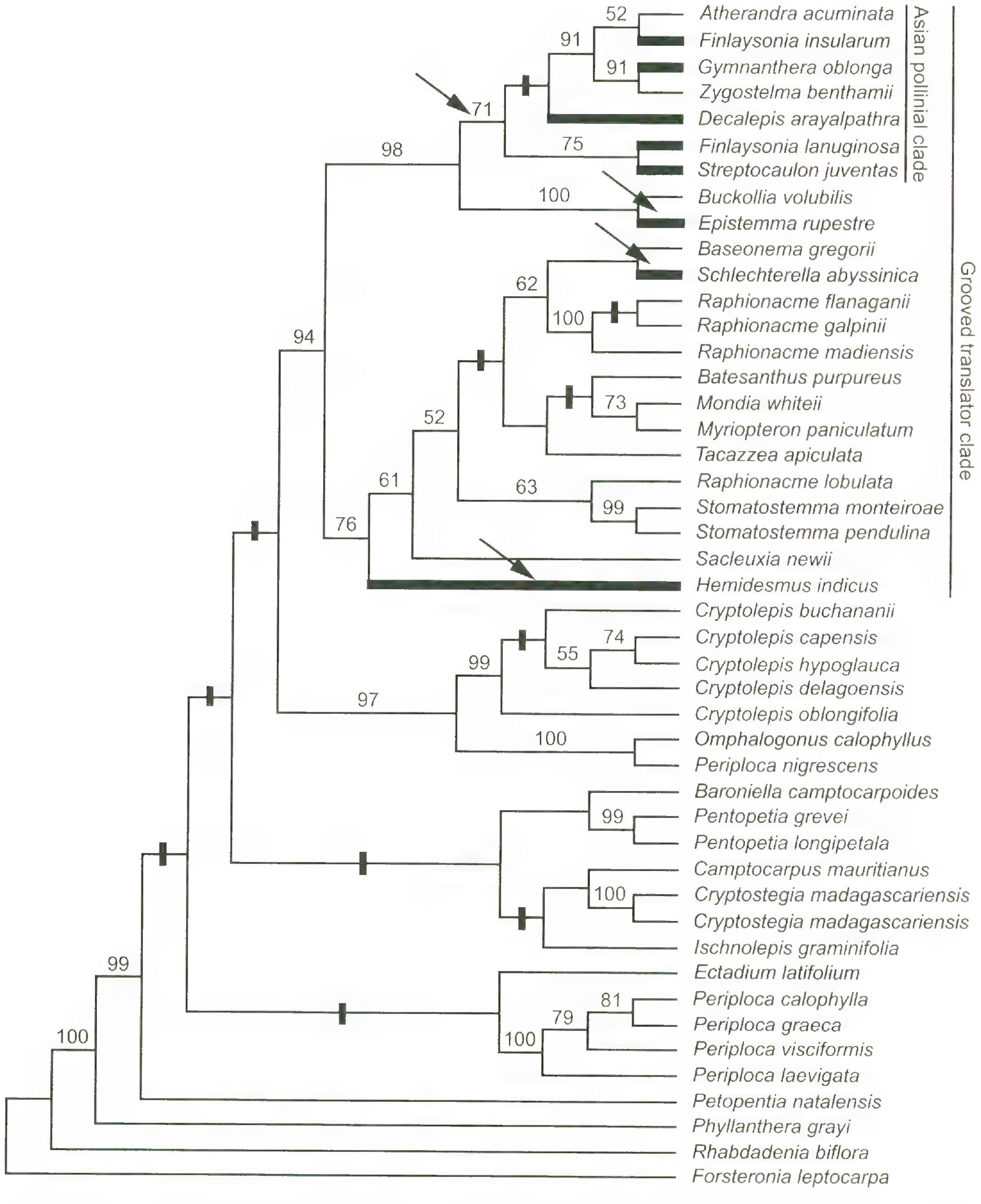

Figure 3. One of 142 equally parsimonious trees (L = 1706, CI = 0.601, RI = 0.620, RC = 0.373) resulting from parsimony analysis of ITS and plastid data combined. Branches in bold indicate pollinial taxa. Numbers above the branches indicate bootstrap values. Putative origins of pollinia using DELTRAN optimization are marked with arrows. Bars indicate branches that collapse in the strict consensus of all shortest trees.

(4) *Epistemma rupestre* (pollinial), with a mix of rounded and elongate rhomboidal tetrads (L/W = 1–2.5); (5) *Hemidesmus indicus* (L.) R. Br. ex Schult. (pollinial), with a mix of rhomboidal, linear, and T-shaped tetrads (L/W = 1.5–3.0); and (6) *Schlechterella abyssinica* (pollinial), with oblong, decussate tetrads (L/W = ca. 2.0). Tetrads were arranged randomly on the translator spoon with the following exceptions: in both pollinial and non-pollinial members of the Asian pollinial clade, tetrads were aligned more or less parallel to one another (in a fan-like fashion when the tetrads were T-shaped) and roughly perpendicular to the translator spoon, and in the pollinial taxon *Schlechterella abyssinica*, tetrads were

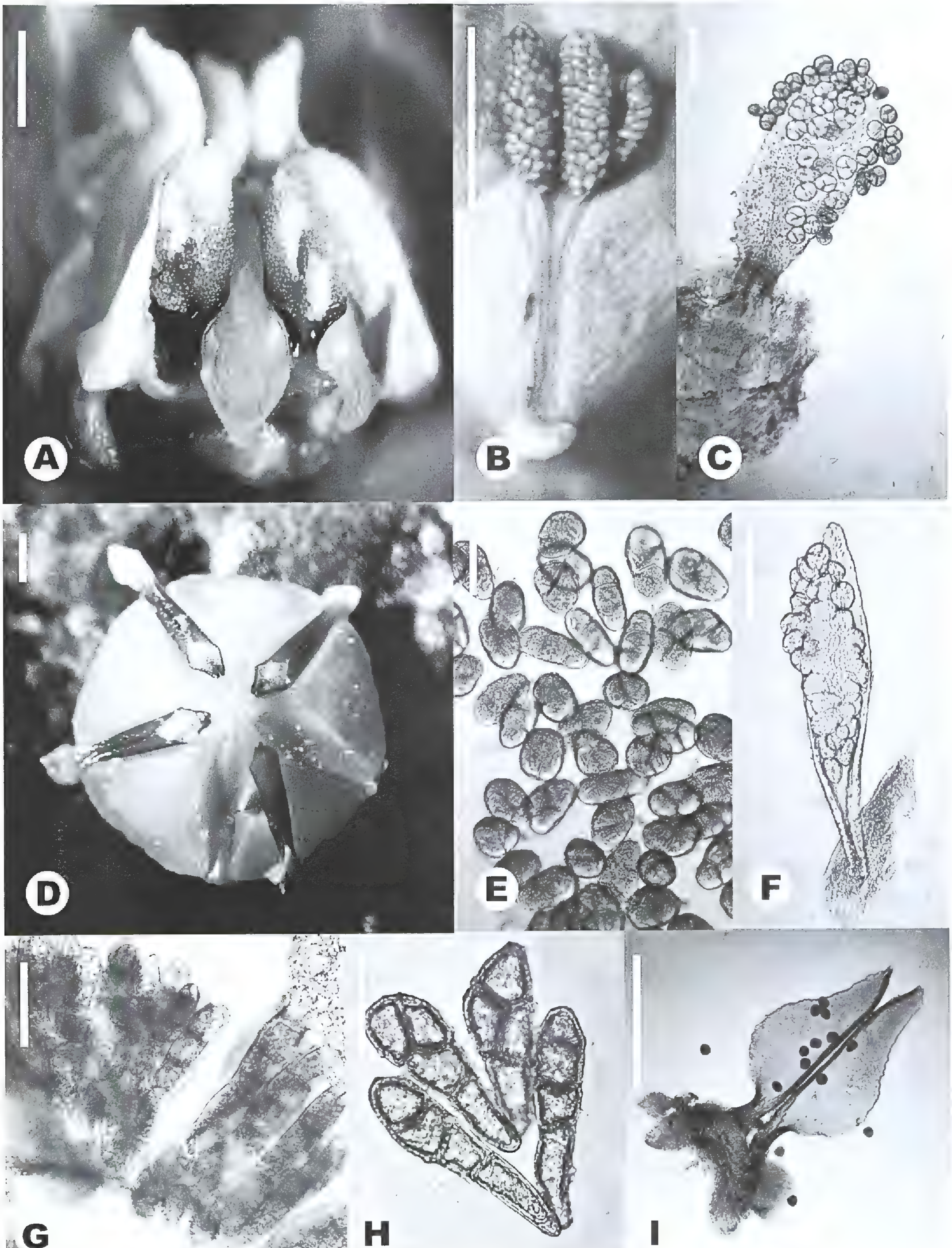

Figure 4. Translators and pollen of Periplocoideae. —A. *Cryptostegia madagascariensis*. Gynostegium with two anthers removed to show translators lying on stylar head. *Ex hort.* (FTG). —B. *Gymnanthera oblonga*. Translator with pollinia in position at anthesis on stylar head. *Forster 6133* (K). —C. *Ectadium latifolium*. Simple translator spoon and rounded tetrads. *Albers 2413* (UBT). —D. *Mondia whiteii*. Stylar head from above, with translators. Note deep longitudinal groove in translator spoon and divided apex. *Ex hort. Ollerton s.n.* —E. *Periploca graeca*. Pollen tetrads. *Endress s.n.* (Z). —F. *Cryptolepis buchananii*. Simple translator spoon with rounded tetrads. *Grierson & Long 2060* (A). –G. *Decalepis hamiltonii*. Elongate, linear tetrads forming pollinia. Note parallel alignment of tetrads. *Nicholson, Saldanha & Ramamoorthy HFP163* (MO). —H. *Finlaysonia insularum*. Four elongate, linear, and T-shaped tetrads removed from pollinium. *Chin 1743* (K). —I. *Stomatostemma monteiroae*. Translator. Note deep groove in translator spoon and rounded tetrads. *Albers & Meve 518* (UBT). Scale bars: A, D = 1 mm; B, I = 500 μm; C, F = 100 μm; E, G, H = 50 μm.

mostly randomly aligned, but at least some were aligned parallel to one another. Tetrad alignment in the pollinial taxa *Epistemma rupestre* and *Hemidesmus indicus* appeared to be random.

Discussion

Phyllanthera: sister to the rest of Periplocoideae?

Phyllanthera grayi is supported as sister to the rest of Periplocoideae in the ITS and total evidence analyses. As currently circumscribed, *Phyllanthera* Blume comprises eight species of Southeast Asian and Australian forest climbers. Forster (1993) placed *Phyllanthera* (then comprised of two species) into synonymy with *Cryptolepis*, recognizing these two taxa plus four *Cryptolepis* species as the subgenus *Phyllanthera*. Venter and Verhoeven (2001) later reinstated the genus *Phyllanthera*, uniting *Cryptolepis* subgenus *Phyllanthera* with the monotypic genus *Pentanura* Blume on the basis of similar floral structures, including a lack of alternipetalous corona lobes, prominent anther apices, and staminal feet swollen and fused around the style.

Phyllanthera grayi (originally described under *Cryptolepis* subgenus *Phyllanthera*) is the sole representative of the genus in our analyses. Its placement at the base of the subfamily comes as a surprise. Endress and Bruyns (2000) described a morphological transformation series in flowers of Periplocoideae leading from (1) *Gymnanthera*-type flowers, with staminal feet running down a long lower corolla tube made up of congenitally fused corolla, stamens, and nectaries (Nishino, 1982; Venter & Verhoeven, 2001), similar to flowers of Rauvolfioideae and Apocynoideae; to taxa in which (2) the lower corolla tube is much shorter, with incompletely fused staminal feet forming a ring around the top of the ovary; to a condition where (3) the staminal feet are completely fused around the style (e.g., *Hemidesmus*); approaching in form the (4) "staminal tube" of Secamonoideae/Asclepiadoideae. Under this scenario, one would expect the early-diverging members of Periplocoideae to have a long corolla tube as in the first condition described above; however, *Phyllanthera*, with staminal feet fused incompletely into a ring around the style, exemplifies the second condition.

Venter and Verhoeven (1997) placed *Phyllanthera* in the tribe Cryptolepideae. The presence of a distinct corolla tube in members of Cryptolepideae led them to hypothesize that this tribe was more advanced than, and perhaps derived from, the tribe Periploceae. They later abandoned this tribal classification based on the results of their own phylogenetic analysis of morphological characters (Venter & Verhoeven, 2001). It is of interest, however, that in their morphology-based phylogenetic analysis *Phyllanthera* again appeared in a derived position (within the "*Atherandra* clade"), in support of the views of Endress and Bruyns (2000), and in conflict with its placement in our DNA-based trees.

At this time, our morphological investigations are still in a preliminary stage. Given the absence of clear morphological characters supportive of *Phyllanthera* in this position, coupled with a lack of support for this relationship in the plastid analyses, we consider the phylogenetic placement of *Phyllanthera* as sister to the remaining genera of Periplocoideae to be an interesting, but still tentative, hypothesis.

A grooved translator clade

A clade containing roughly half of the species sampled (23 of 43 ingroup taxa) appears in both the ITS (Fig. 1, BS 90) and total evidence analyses (Fig. 3, BS 94). This clade, comprising African and Asian taxa, includes the eight pollinial exemplars. Most taxa in this clade have pollen translators with a prominent groove (corresponding to a ridge in the stylar head where the translator is formed) running transversely along the spoon (Fig. 4D, I). Often there is a notch at the top of the spoon, which may extend longitudinally down the spoon, partially dividing it into two halves. Exceptions are the genus *Raphionacme*, in which the groove is absent or shallow, and *Baseonema gregorii* Schltr. & Rendle, *Sacleuxia newii* (Benth.) Bullock, and *Schlechterella abyssinica*, whose translators have a shallow groove. These likely represent reversals.

Taxa outside of this grooved translator clade bear translators with flattened spoons and rounded apices (e.g., *Ectadium latifolium*; Fig. 4C); in some cases (e.g., *Petopentia natalensis*) the central axis of the spoon is slightly thinner. We note that the Malagasy taxa sampled in this study (representing all five Malagasy genera) fall outside of the grooved translator clade. Arekal and Ramakrishna (1980: 430) observed that translator spoons in *Decalepis* and *Hemidesmus* (both within the grooved translator clade) had "a median partition wall, dividing it into two parts, each of which receives pollen tetrads of the nearest anther lobe." They interpreted these translators as more specialized than the un-grooved translators of *Cryptolepis buchananii* (Fig. 4F) and *Cryptostegia madagascariensis* (Fig. 4A), neither of which appeared in the grooved translator clade in our analyses. Safwat (1962: 113) noted that "the genus *Cryptolepis* is considered to be rather primitive because of the poorly developed translators, a fact which led R. Brown to include it within the Apocynaceae."

AN ASIAN POLLINIAL CLADE

There is moderate support for a clade of seven Asian species (five of which bear pollinia) in the ITS (Fig. 1, BS 72), plastid (four taxa in the plastid data set, Fig. 2, BS 81), and total evidence analyses (Fig. 3, BS 71). In the ITS and total evidence analyses, this clade appears within the grooved translator clade. Members of the Asian pollinial clade appear in all three tribes of Venter and Verhoeven (1997) and did not form a clade in their morphology-based cladistic analysis (2001). This conclusion of Verhoeven and Venter (1997, 2001) is understandable; these are, morphologically, seemingly disparate taxa with little uniting them beyond the presence of pollinia in most. *Gymnanthera oblonga*, for example, has flowers up to 17 mm long, a long corolla tube, and squat, transverse-triangular coronas (Forster, 1991). In contrast, *Zygostelma benthamii*, also in the Asian pollinial clade (sister to *G. oblonga*, Fig. 3, BS 91), has flowers ca. 6 mm long, a short corolla tube, and unique, curiously shaped coronas that resemble a pair of ram's curved horns. The Asian pollinial clade exemplifies the wide range of morphological variation found in closely related taxa throughout the subfamily; this variability, in part, explains the large number of monotypic and ditypic genera that have been recognized in Periplocoideae, as well as the difficulty in assessing relationships on the basis of morphology alone.

Pollen and translators offer clues to intergeneric relationships in Periplocoideae. In most taxa, pollen tetrads are round or short-oblong and arranged randomly on the translator spoon (Fig. 4A, C, I). In contrast, we observed that both pollinia and tetrad-bearing members of the Asian pollinial clade have elongate pollen tetrads that are aligned roughly parallel to one another (sometimes the tetrads are wider at the distal end; this results in a fanlike arrangement) and more or less perpendicular to the translator spoon (Fig. 4B, G, H). Tetrad shape apparently does show some homoplasy in Periplocoideae. We observed a mix of tetrad shapes including elongate forms in *Periploca graeca* and *Petopentia natalensis* and in the three pollinial taxa appearing outside of the Asian pollinial clade in our analyses: *Epistemma rupestre* has rounded to oblong tetrads, *Hemidesmus indicus* has a mix of shapes, and *Schlechterella abyssinica* has oblong, decussate tetrads. Thus, with a few exceptions, there is an intriguing trend from more or less rounded tetrads in non-pollinial Periplocoideae toward the elongation of pollen tetrads in the pollinial taxa and in the tetrad-bearing members of the Asian pollinial clade.

The genus *Atherandra* Decne. has been documented as having pollen arranged in tetrads (Venter & Verhoeven, 2001). Prior to including *Atherandra acuminata* in our phylogenetic analyses, we noted that in this species, translator shape and the shape and arrangement of pollen tetrads is similar to that of species in the Asian pollinial clade: the tetrads are elongate, aligned parallel to one another, and roughly perpendicular to the translator spoon. Based solely on this, we speculated that *A. acuminata* would group within the Asian pollinial clade, despite having tetrads rather than pollinia. Indeed, when included in our analysis, *A. acuminata* grouped with the Asian pollinial taxa, with good support (Fig. 3, BS 71).

A MALAGASY CLADE?

Five Periplocoideae genera are endemic to Madagascar and the Mascarene Islands: *Baroniella*, *Camptocarpus*, *Cryptostegia*, *Ischnolepis*, and *Pentopetia*. The relationship of these Malagasy genera to each other and to other Periplocoideae is poorly resolved in the ITS and combined analyses, but in the combined plastid analysis (*Baroniella* is missing from the plastid data sets) they form a well-supported clade (Fig. 2, BS 82). This is in accordance with the findings of Meve and Liede (2004); their analyses of plastid data supported a Malagasy clade (*Camptocarpus–Ischnolepis–Pentopetia*), a relationship not recovered from their analyses of ITS and total evidence data sets. The hypothesis of a Malagasy clade is compelling; however, at this time there is insufficient support for this grouping.

MONOPHYLY OF GENERA AND THE GENUS *PERIPLOCA*

Our analyses support the monophyly of *Stomatostemma*, *Pentopetia*, and *Cryptolepis* (Figs. 1, 3). Both species of *Stomatostemma* were sampled; *Pentopetia* and *Cryptolepis*, however, are fairly large genera (21 and 27 species, respectively); thus, further taxon sampling is warranted in order to confirm our results. *Periploca*, the type genus of the subfamily, occurs in a variety of habitats in Asia and Africa and extends into Europe. The genus exhibits a wide range of growth types, from forest climber to scrambling or erect shrub to geophytic herb (Browicz, 1966; Venter, 1997). Venter (1997) delimited two sections in *Periploca* based on the presence (section *Periploca*, further divided into three subgroups, or series) or absence (section *Monocoronata*) of basally tri-segmented corona lobes. In our analyses, which included exemplars from all four subgroups, representatives of section *Periploca* (*P. calophylla*, *P. graeca*, *P. visciformis*, and *P. laevigata*) form a well-supported clade. *Periploca nigrescens*, in contrast (representing section *Monocoronata*, the other main group delimited

by Venter), is well supported as sister to *Omphalogonus calophyllus* (Fig. 3, BS 100); together, these two taxa are sister to *Cryptolepis*.

The genus *Periploca* is quite distinctive: nine of 14 species have a dark fleshy center on the adaxial corolla lobes, often surrounded by hairs or with a white papillose spot in the center. These corolla features, unique to *Periploca* and perhaps synapomorphic, are not present in *P. nigrescens* or other members of section *Monocoronata*. Venter and Verhoeven (1997) hypothesized that section *Monocoronata* represented the least modified of *Periploca* species and further speculated that *P. nigrescens* was most likely not closely related to other members of that section. Inclusion of additional exemplars from *Periploca* section *Monocoronata* is planned in order to further test the monophyly of the genus.

OMPHALOGONUS CALOPHYLLUS–PERIPLOCA NIGRESCENS

Periploca nigrescens and *Omphalogonus calophyllus* form a well-supported clade in the ITS and total evidence analyses (Figs. 1, 3, BS 100). It is difficult to distinguish between these two species based solely on vegetative characters; both form large lianas with leaves of similar shape, texture, and appearance, and the plants are black when dried, a condition not present in other Periplocoideae. They are so similar in vegetative form that Bullock (1961) combined *Periploca nigrescens–Parquetina gabonica* Baill.–*Omphalogonus calophyllus* into a single species, *Parquetina nigrescens* (Afzel.) Bullock. Floral morphology in these taxa does, however, differ: the flowers of *P. nigrescens* are rotate, the gynostegium is exserted, and the anthers are large, while in *Omphalogonus*, flowers are campanulate with an enclosed gynostegium and less-conspicuous anthers. Recognizing these differences in floral morphology, Venter and Verhoeven (1996) later reinstated *O. calophyllus* and *P. nigrescens* as distinct species in two different genera, and in 1997 even placed them in separate tribes. Our findings that *P. nigrescens* and *O. calophyllus* are well supported as sister taxa illustrate the importance of considering both floral and vegetative features when assessing relationships within this complex group.

A POLYPHYLETIC *FINLAYSONIA*

Venter and Verhoeven (2001) placed several monotypic and ditypic Asian genera into synonymy under the previously monotypic *Finlaysonia* due to similarities in floral form (the gynostegium exposed from a rotate or saucer-shaped corolla) and because all have pollen fused into pollinia. We included in our study two *Finlaysonia* species: *F. insularum* (=*Gymnanthera insularum* King & Gamble, later *Meladerma insularum* (King & Gamble) Kerr.) and *F. lanuginosa* (=*Gongylosperma lanuginosum* Ridley). We were unable to obtain suitable material for DNA sequencing of *F. obovata* Wall., the generic type. Our analyses did not support the monophyly of *Finlaysonia* s.l.: in the ITS and combined analyses, *F. insularum* appears in a clade with *Atherandra acuminata–Gymnanthera oblonga–Zygostelma benthamii* (Figs. 1, 3), while *F. lanuginosa* pairs with *Streptocaulon juventas*. The plastid data set also strongly supports the relationship between *F. lanuginosa* and *S. juventas* (Fig. 2). *Finlaysonia lanuginosa* has a densely pubescent abaxial leaf surface similar to that seen in *Streptocaulon* species, in contrast to the glabrous condition observed in other *Finlaysonia* species examined. *Finlaysonia* as currently circumscribed is an amalgamation of six smaller genera recently placed in synonymy (Venter & Verhoeven, 2001). We hope to include as many of these species as possible in future analyses of both molecular and morphological data.

RAPHIONACME LOBULATA RENDERS *RAPHIONACME* NON-MONOPHYLETIC

In a cladistic analysis of molecular data, which included 17 species in nine genera of Periplocoideae, Meve and Liede (2004) found good support for the monophyly of *Raphionacme*, a genus of 37 species. The inclusion of *R. lobulata* in our analyses rendered *Raphionacme* non-monophyletic; *R. lobulata* is not allied with other *Raphionacme* exemplars and is instead supported as sister to *Stomatostemma* (Figs. 1, 3). Venter and Verhoeven (1988) originally placed *R. lobulata* in the genus *Raphionacme* based on similarities in floral features such as corolla structure and the position of stamens and corona lobes on the corolla tube. They noted pollen grains with eight pores in *R. lobulata*. Most Periplocoideae have four to six pores per pollen grain; *Raphionacme*, *Schlechterella*, and *Baseonema* Schltr. & Rendle have eight to 16. Venter et al. (2006) later noted that vegetatively *R. lobulata* more closely resembles *Stomatostemma* (similar leaves and habit, and similar strings of spherical tubers on their roots) and *Batesanthus* N. E. Br. (similar fleshy interpetiolar stipules) than *Raphionacme*, and a re-investigation of pollen in this taxon showed four to six pores per grain. Our results, which indicate a close relationship between *R. lobulata* and *Stomatostemma*, are in accordance with these observations. Here again we see that vegetative and pollen characters can be more informative than gross floral morphology in assessing relationships in Periplocoideae.

EVIDENCE FOR MULTIPLE ORIGINS OF POLLINIA

We mapped the presence of pollinia onto one of the shortest trees from the total evidence analysis; our results indicate either three or four separate origins of pollinia in Periplocoideae, each of which require four evolutionary changes. Under DELTRAN optimization (Fig. 3), pollinia are hypothesized to have originated four times: once in the common ancestor of the Asian pollinial clade, two more times (separately) in the African genera *Epistemma* and *Schlechterella*, and a fourth time in *Hemidesmus*, a monotypic genus from India. Under ACCTRAN optimization, pollinia are hypothesized to have evolved three times: in the common ancestor of the Asian pollinial–*Epistemma*–*Buckollia* Venter & R. L. Verh. clade, again in *Schlechterella*, and a third time in *Hemidesmus*. Thus, it is equally parsimonious to view the pollinia of *Epistemma* as homologous with those of the Asian pollinial clade (in which case *Buckollia* represents a reversal to tetrads) or as representing yet a fourth separate origin of pollinia in the subfamily. These results support the hypothesis of Fishbein (2001) that pollinia evolved more than once in Periplocoideae, and more specifically, the hypotheses of Venter and Verhoeven that pollinia in Asian and African Periplocoideae evolved polyphyletically (1997) and independently (2001).

Our preliminary observations suggest that pollinial form varies in a manner consistent with phylogenetic pattern and is suggestive of multiple origins of pollinia within the subfamily. The pollinia of *Schlechterella* are very different from those found in other pollinial taxa. The most striking difference can easily be seen in SEM images: in all other species with pollinia, the distal tetrad walls are inaperturate or vestigially porate (Verhoeven & Venter, 2001), while in *Schlechterella* (Verhoeven & Venter, 1998b), the pollen tetrads have pores on both proximal and distal walls. The proximal walls of a pollinium are defined as walls between adjacent tetrads; distal walls are on the outside of the pollinium (Verhoeven & Venter, 1994b, 1998a).

We observed elongate pollen tetrads aligned parallel to one another and relatively perpendicular to the translator spoon in members of the Asian pollinial clade (pollinial and tetrad-bearing taxa). This is in contrast to the randomly aligned tetrads of *Schlechterella* and non-pollinial taxa. We were unable to determine the orientation of tetrads to the translator spoons in *Hemidesmus* and *Epistemma*, but additional study is planned.

In Periplocoideae, pollinia are made up of loosely agglutinated tetrads (Verhoeven & Venter, 1998a). Tetrads may be fused (tectum and granular layers) in places where the pores of adjacent tetrads are touching. Fusion between tetrad pores has not been observed in *Hemidesmus*. Verhoeven and Venter (1998a) observed wavy proximal tetrad walls in the pollinia of *Hemidesmus* and *Schlechterella* and postulated that this feature may assist in holding the tetrads together. These wavy tetrad walls were not observed in members of the Asian pollinial clade, *Epistemma*, or other Periplocoideae.

General Conclusions

The use of molecular evidence to elucidate evolutionary relationships can be particularly crucial in groups that exhibit a complex and widely varying array of floral forms such as Periplocoideae. The results of our analyses do not always agree with past generic or tribal level classifications; this may stem, at least in part, from an emphasis on rather homoplasious floral features by previous systematists. In agreement with previous studies, our results show that pollinia in Periplocoideae are likely non-homologous with those of Asclepiadoideae and Secamonoideae and support at least three origins of pollinia in Periplocoideae. We hope to clarify relationships and elucidate patterns of character evolution in this fascinating group through further sampling of taxa from throughout the subfamily, by completing sequencing of the plastid regions used in this study, and with more in-depth morphological investigations.

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PHYLOGENETIC RELATIONSHIPS BETWEEN DERIVED APOCYNACEAE S.L. AND WITHIN SECAMONOIDEAE BASED ON CHLOROPLAST SEQUENCES[1]

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ABSTRACT

Phylogenetic relationships between derived subfamilies of Apocynaceae s.l. as well as within the subfamily Secamonoideae have been inferred using maximum parsimony and Bayesian analyses. Characters are provided by four chloroplast sequences: *matK*, *trnT-L* and *trnL-F* spacers, and *trnL* intron. Phylogenies from both analyses show a sister group relationship between a clade of derived Apocynoideae included in the subtribe Baisseinae (tribe Apocyneae) and another clade including representatives from the Secamonoideae and Asclepiadoideae. Within the Secamonoideae, two genera, *Pervillaea* Decne. and *Secamonopsis* Jum., are strongly supported as monophyletic, whereas the monophyly of the largest genus, *Secamone* R. Br., remains uncertain. Palynological and morphological characters are discussed for the Malagasy *Secamone* in relation to one of the most parsimonious molecular trees.

Key words: Adaptive radiation, Apocynaceae, Madagascar, *matK*, phylogeny, Secamonoideae, *trnL*, *trnL-F*, *trnT-L*.

The Secamonoideae (Endlicher, 1838) are a small subfamily of the former Asclepiadaceae s. str., now included in Apocynaceae s.l. (Endress & Bruyns, 2000). In the treatment of Endress and Bruyns, nine genera were accepted: *Calyptranthera* Klack., *Genianthus* Hook. f., *Goniostemma* Wight, *Pervillaea* Decne., *Rhynchostigma* Benth., *Secamone* R. Br., *Secamonopsis* Jum., *Toxocarpus* Wight & Arn., and *Trichosandra* Decne. *Goniostemma*, however, is of uncertain taxonomic validity (Venter & Verhoeven, 1997), and the monotypic African genus *Rhynchostigma* has recently been put into synonymy under *Secamone* (Klackenberg, 2001). The Secamonoideae have fewer than 200 species, restricted mostly to the Old World tropics. *Secamone* is the largest genus, with more than 90 species that occur mainly in Madagascar and the Mascarene Islands (Klackenberg, 1992a), Africa (Goyder, 1992; Klackenberg, 2001; Bruyns, 2004), Australia, and Asia (Forster & Harold, 1989; Klackenberg, 1992b). *Toxocarpus*, with more than 50 species, occurs mainly in Asia, as does *Genianthus*, with 16 species (Klackenberg, 1995a). The four other genera, *Pervillaea* (Klackenberg, 1995b, 2005a), *Secamonopsis* (Civeyrel & Klackenberg, 1996), *Calyptranthera* (Klackenberg, 1996a, b, 1997a), and *Trichosandra* (Friedman, 1990), are restricted to Madagascar or to the Mascarene Islands, with fewer than 10 species each. The main center of biodiversity for the subfamily is Madagascar (Klackenberg, 1992a), where about half of the known species and genera occur, followed by Southeast Asia, and then Africa (Klackenberg, 1992b, 1995a, 2001).

Recent studies appraising the phylogeny and systematics of the Apocynaceae s.l. have shown the Secamonoideae to be a monophyletic sister group to the Asclepiadoideae (Sennblad & Bremer, 1996, 2000, 2002; Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Fishbein, 2001; Potgieter & Albert, 2001; Lahaye et al., 2005). However, the sister group to the larger clade including Secamonoideae and Asclepiadoideae is still not unambiguously identified. Molecular phylogenies based on the chloroplast gene *matK*

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have shown the Periplocoideae to be the sister group to the Asclepiadoideae–Secamonoideae clade (Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Fishbein, 2001: Lahaye et al., 2005), and the tribe Apocyneae (Apocynoideae) to be the sister group to the former Asclepiadaceae (Civeyrel et al., 1998; Civeyrel & Rowe, 2001). This has not been the case in studies based on the chloroplast gene *rbcL* (Sennblad & Bremer, 2000, 2002) and the intron *trnL* combined with the spacer *trnL-F* (Potgieter & Albert, 2001), in which the tribe Apocyneae appeared polyphyletic and one of its members, *Baissea* A. DC., was sister to a group including Secamonoideae and Asclepiadoideae, though only weakly supported.

The most inclusive phylogeny of the Secamonoideae to date (Lahaye et al., 2005) was based on the two chloroplastic sequences *matK* and *trnT-L* (2543 characters, 9% parsimony informative) and included 26 taxa from three genera related to the Secamonoideae (around 15% of the total species in the subfamily) from Madagascar and the Mascarene Islands. Although this study included a large sampling combined with a high number of characters, it did not resolve the internal relationships between genera. *Pervillaea* and *Secamonopsis* were strongly supported as monophyletic, but a polytomy between these two genera, a second clade consisting of *Secamone oleaefolia* Decne. from coastal eastern Madagascar and *S. volubilis* (Lam.) Marais from La Réunion, and a third clade of other Malagasy *Secamone*, made the monophyly of this last genus uncertain. The study further indicated that several clades within Malagasy *Secamone* were congruent with morphological (Klackenberg, 1992a) or palynological (Civeyrel, 1994) observations.

The Secamonoideae are the central focus of this paper. The principal aims are to clarify relationships between the Secamonoideae and derived Apocynaceae s.l. and to increase the resolution of the internal phylogenetic relationships within the subfamily. We combined previously published sequences from the two chloroplast regions *matK* and *trnT-L* (Endress et al., 1996; Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Lahaye et al., 2005) with two other chloroplast regions, the *trnL* intron and the *trnL-F* spacer. For each new region, six previously published sequences (Potgieter & Albert, 2001; Liede & Täuber, 2002) have been used in the present study. Three new taxa forming the subtribe Baisseinae (Apocyneae) have been added to the 45 taxa included in the analysis of Lahaye et al. (2005).

The *trnL* intron and the *trnL-F* spacer are noncoding regions of chloroplast DNA (cpDNA) for which universal primers exist (Taberlet et al., 1991). Those plastid regions have proved useful for resolving infrafamilial relationships among different groups of plants and have previously been used to resolve relationships within the Apocynaceae s.l. (Potgieter & Albert, 2001) and the Asclepiadoideae (Liede & Täuber, 2000, 2002; Liede, 2001; Liede et al., 2002, 2005; Meve & Liede, 2002; Rapini et al., 2003; Yamashiro et al., 2004). It is thought useful to combine these plastid regions, because the relatively low variation of the *trnL* intron (Taberlet et al., 1991) is supposed to structure the topology, whereas the relatively high variation of the *trnL-F* (Taberlet et al., 1991) spacer is supposed to resolve interspecific relationships.

Material and Methods

Ingroup and Outgroup Sampling

Taxa were sampled from three genera of Secamonoideae in Madagascar: *Pervillaea*, *Secamonopsis*, and *Secamone*. Appendix 1 provides information on the taxa, voucher specimens, and GenBank accession numbers for all sequences. The three subfamilies Secamonoideae, Asclepiadoideae, and Periplocoideae, together with three taxa of derived Apocynoideae, constitute the ingroup. The outgroup taxa were selected from Apocynoideae and Rauvolfioideae. To the *matK* and *trnT-L* sequences published previously (Endress et al., 1996; Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Lahaye et al., 2005), we added three new sequences of *matK* and 42 new sequences each of *trnL-F* and *trnL* (Appendix 1).

DNA Extraction, Amplification, and Sequencing

DNA extraction, amplification, and sequencing for sequences previously published follow Lahaye et al. (2005); only new protocols used for this study will be described here. Total DNA used for the new sequences was extracted from 20–40 mg of herbarium material by beating the material in tubes containing plastic beads, and then treating samples with the Qiagen DNAeasy plant kit (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) amplifications were conducted using 2 µl of total DNA.

The chloroplast gene *matK* was PCR amplified in two crossing parts using the primer pairs *matK* 8F and *matK* 829 R, and *matK* 681F and *matK* 1628R (Endress et al., 1996; Civeyrel et al., 1998). The *trnL* intron and *trnL-F* spacer of the chloroplast genome was PCR amplified using the primer pairs c and d and e and f, respectively, designed by Taberlet et al. (1991). These PCR amplifications were carried out using the kit ready-to-go PCR beads (Amersham Biosciences, U.S.A.). These beads containing a premix with 2.5 units of DNA polymerase were reconstituted

to 25 µL final volume in which the concentration of each dNTP is 200 µM, with 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, and 1.5 mM $MgCl_2$. To amplify the inferred sequences, the same protocol for thermal reactions was carried out, as follows: (1) one initial cycle of 5 min. at 95°C to ensure denaturation of the double-stranded template DNA; (2) three cycle steps repeated 40 to 44 times: denaturation at 95°C for 15 sec., annealing at 50°C for 1 min., and extension at 72°C for 1 min. 30 sec.; and (3) a final extension step at 72°C for 5 min. to complete unfinished DNA strands. The PCR reactions were purified using the QIAquick spin columns following the manufacturer's protocols (Qiagen, Helden, Germany) and sequenced at the Molecular Systematic Laboratory in Stockholm (Swedish Museum of Natural History) using the Thermo Sequenase Sequencing kit from Amersham Pharmacia Biotech. Sequencing reactions were electrophoresed on 6% Long Ranger gels on a Pharmacia ALF-express automated sequencer. Both strands of DNA were sequenced using the same primers as those used for amplification.

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

New data files for *matK*, *trnL*, and *trnL-F* sequences were assembled and edited using the Staden Package (Staden, 1996). Ambiguous positions were coded using appropriate IUPAC (International Union of Pure and Applied Chemistry) ambiguity symbols so as to maximize retention of information. We eliminated the first 50 bases at the 5′ and 3′ ends of *matK*, the 5′ end of *trnL*, and the 3′ end of *trnL-F*, because the nucleotide sequences were not completely resolved near the primers and were not confirmed by overlapping sequences. Sequences were initially aligned using ClustalX version 1.8 (Thompson et al., 1997), and the alignment was improved visually. Indels found in the alignment of *matK*, *trnT-L*, *trnL*, and *trnL-F* were included in the analysis but not coded. Their phylogenetic value will be discussed for the Secamonoideae.

Phylogenetic analyses were performed using maximum parsimony (MP), and for comparison, a Bayesian method of phylogenetic inference (Mr. Bayes, ver. 3.1; Huelsenbeck & Ronquist, 2001) was employed. Parsimony analysis was conducted assuming unordered character states. Gaps were treated as missing data. Parsimony analyses were completed using the software package PAUP* version 4.0b10 (Swofford, 2002) with a heuristic search strategy. Each data matrix was analyzed using 1000 replicates of random taxon addition holding 10 trees per replicate to find islands of equally parsimonious trees with tree bisection-reconnection (TBR) on. Complete swapping was performed on all trees accumulated in the replicates (which should have found all trees at that length) with a tree limit of 40,000 and MULTREES on. Rounds of successive approximation weighting (hereafter SW; Farris, 1969; see also Civeyrel et al., 1998) were added, with characters re-weighted according to their rescaled consistency index (RC), based on best fit characters. Three re-weighting steps were repeated until the tree length remained unchanged over two successive iterations. To evaluate clade support, bootstrap (BS) analyses (Felsenstein, 1985) were performed using a full heuristic search with 1000 replicates, retaining BS frequencies from 1% to 100%, which were compatible with the 50% majority rule consensus tree, simple sequence addition, sampling characters with equal probability but applying weight (from SW), TBR, MULTREES on, and a tree limit of 1000. Bootstrap percentages (BP) are referred to as high (85%–100%), moderate (75%–81%), or low (50%–74%). All illustrated trees use ACCTRAN optimization. The base weight of 1000 applied in SW was removed for tree presentation.

The incongruence length difference (ILD; Farris et al., 1994) test was employed to detect incongruence among the data sets using partition homogeneity test in PAUP*. The partition *matK* versus *trnT-L* versus *trnL* versus *trnL-F* homogeneity test was performed using 750 replicates on parsimony informative characters (Cunningham, 1997), TBR branch-swapping, 100 replicates of random addition sequence holding one tree per replicate, and MULTREES on (MAXTREE = 1000, and other default settings).

The Bayesian analysis was conducted using a general time reversible (GTR + I + G) model determined to be the best fit model for our data sets using Modeltest ver. 3.7 (Posada & Crandall, 1998), with gamma distributed rate variation across sites and an initial estimate of equal base frequencies (Jukes & Cantor, 1969). Two Markov Monte Carlo chains were run simultaneously starting from random trees. The analysis was run for 2,000,000 generations, sampling a tree every 500 generations with a burn-in value (number of trees ignored) of 1000 (25% of total trees saved). The 3001 remaining trees retained after reaching the stationary phase (after ca. 30,000 generations) were used to build a majority rule consensus tree with PAUP*, which is shown as a cladogram with posterior probability values in Figure 1.

RESULTS

DNA MATRIX FEATURES AND SEQUENCE DIVERGENCE

Length variation of the inferred sequences ranged from 590 (*Ischnolepis graminifolia* (Costantin &

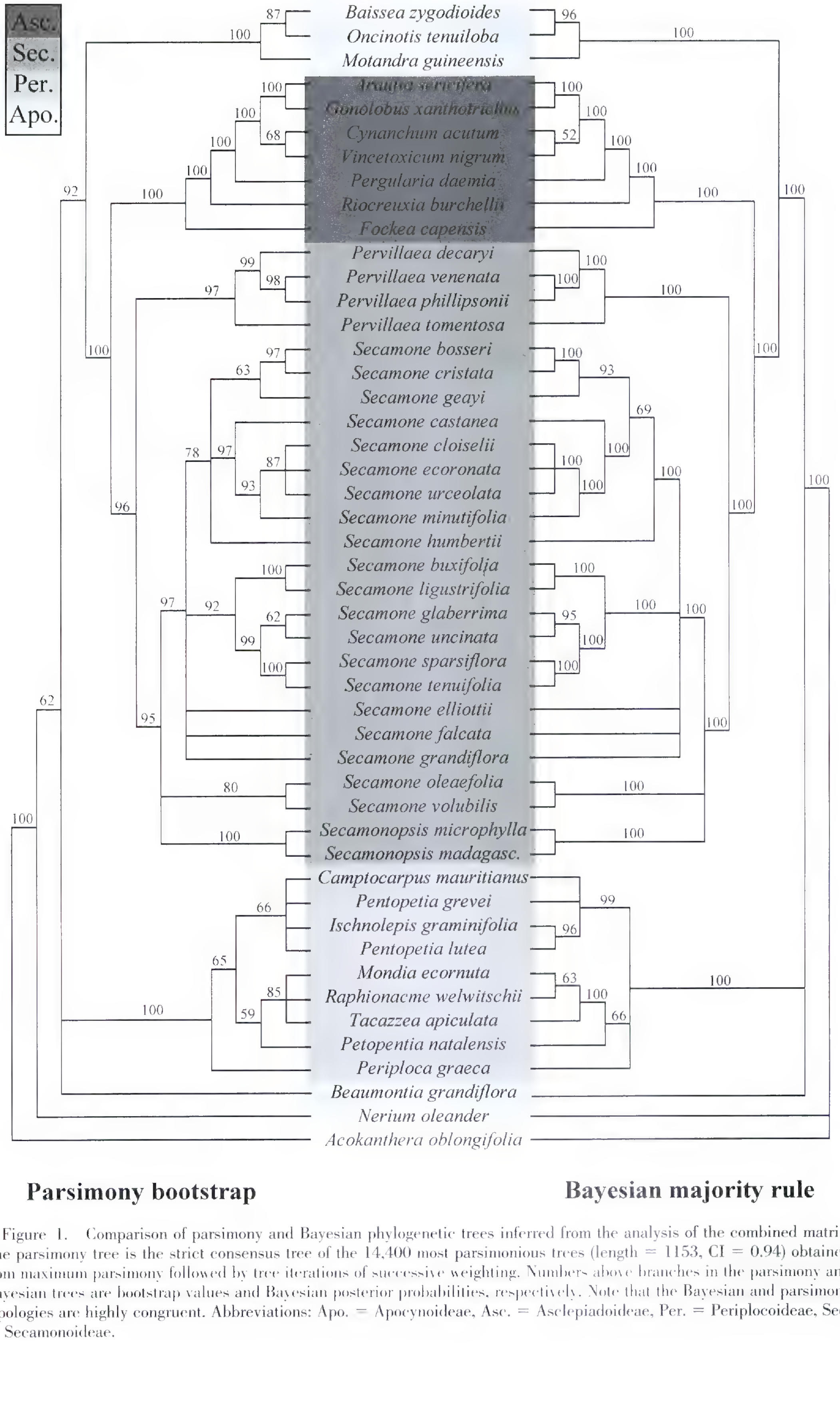

Figure 1. Comparison of parsimony and Bayesian phylogenetic trees inferred from the analysis of the combined matrix. The parsimony tree is the strict consensus tree of the 14,400 most parsimonious trees (length = 1153, CI = 0.94) obtained from maximum parsimony followed by tree iterations of successive weighting. Numbers above branches in the parsimony and Bayesian trees are bootstrap values and Bayesian posterior probabilities, respectively. Note that the Bayesian and parsimony topologies are highly congruent. Abbreviations: Apo. = Apocynoideae, Asc. = Asclepiadoideae, Per. = Periplocoideae, Sec. = Secamonoideae.

Table 1. Characteristics of the *matK*, *trnT-L*, *trnL*, *trnL-F*, and combined sequences* constituting the matrices.

	matK	*trnT-L*	*trnL*	*trnL-F*	Combined matrix
Length range (bp)	590–1505	262–751	474–509	358–454	2153–3414
Aligned length (bp)	1547	989	537	484	3557
No. of parsimony-uninformative sites	262	121	49	66	498
No. of parsimony-informative sites	162	76	34	37	309
No. of constant sites	1123	792	454	381	2750
% parsimony-informative sites	10.47	7.68	6.33	7.64	8.69
No. of indels (total)	17	76	22	31	146
No. of autapomorphic indels	10	45	12	21	88
Ingroup sequence divergence (%)	0.06–8	0–8	0–6.7	0–8.6	0.03–7.5
Ingroup/outgroup sequence divergence (%)	3.2–8.5	1.4–8.3	1.2–6.8	1.2–7.9	2.4–7.9
G+C content range (%)	31.8–35.4	19.4–26.3	35.8–38.1	34.2–38.2	31.8–35.6
Transitions (total)	24,446	7374	5202	5063	42,085
Transvertions (total)	24,259	10,312	4077	6761	45,409
Transitions/transvertions	1.01	0.72	1.28	0.75	0.93

* Sequence divergence was estimated using uncorrected pairwise distances.

Gallaud) Klack., incomplete sequence) to 1505 bp for *matK*, 262 (*Secamone glaberrima* K. Schum., incomplete sequence) to 751 bp for *trnT-L*, 474 to 509 bp for *trnL*, 358 (*S. castanea* Klack., incomplete sequence) to 454 bp for *trnL-F*, and 2153 to 3414 bp for the combined matrix (Table 1). Aligned sequence lengths for these same regions were 1547, 989, 537, 484, and 3557 bp, respectively. Alignments showed 17 indels in the *matK* matrix, 76 indels in *trnT-L*, 22 in *trnL*, 31 in *trnL-F*, and 146 in the combined matrix. The G+C content ranged from 31.8% to 35.4% in *matK*, 19.4% to 26.3% in *trnT-L*, 35.8% to 38.1% in *trnL*, 34.2% to 38.2% in *trnL-F*, and 31.8% to 35.6% in the combined matrix. Sequence divergence between ingroup and outgroup taxa ranged from 3.2% to 8.5% in *matK*, 1.4% to 8.3% in *trnT-L*, 1.2% to 6.8% in *trnL*, 1.2% to 7.9% in *trnL-F*, and 2.4% to 7.9% in the combined matrix. Sequence divergence among ingroup taxa ranged from 0.06% to 8% in *matK*, 0% to 8% in *trnT-L*, 0% to 6.7% in *trnL*, 0% to 8.6% in *trnL-F*, and 0.03% to 7.5% in the combined matrix.

PHYLOGENETIC RESULTS

The number of parsimony-informative sites (excluding indels) detected in the sequences inferred was 162 in the *matK* region (10.47% of the total number of sites), 76 in *trnT-L* (7.6%), 34 in *trnL* (6.34%), 37 in *trnL-F* (7.64%), and 309 in the combined matrix (8.69%) (Table 1). The result of the ILD test was significant ($P = 0.02$), indicating that we could reject the null hypothesis of data set homogeneity ($P < 0.05$) (Farris et al., 1994). The low number of informative sites within both the *trnL* and *trnL-F* matrices relative to the number of taxa creates considerable polytomies in the reconstruction and heterogeneity between the phylogenetic information given by the data sets. Moreover, there is no apparent reason to suspect incongruence among different regions of a uniparentally inherited and non-recombining genome. Thus, the following results and comments will be based only on the phylogenetic analyses of the combined matrix.

The successive weighting analyses followed the first step of maximum parsimony of the combined matrix with all characters included. This reduced the number of trees from 40,000 to 14,400 (1153 steps without weights and 797,692 weights included) after three iterations and increased the consistency index (CI) from 0.80 to 0.94 (0.62 to 0.84 uninformative characters excluded), the retention index (RI) from 0.83 to 0.95, and the RC from 0.67 to 0.90. Topologies of the two strict consensus trees resulting from complete swapping and successive weighting, respectively, are almost identical. The relationships within the Periplocoideae are better resolved by SW analysis than complete swapping, with three more branches resolved. On the consensus tree obtained after SW (Fig. 1), the monophyly of each of the three subfamilies of the Asclepiadaceae s. str. is strongly supported with a BS of 100% for the Periplocoideae, 100% for the Asclepiadoideae, and 96% for the Secamonoideae. Furthermore, representatives from the Secamonoideae form, together with their sister group Asclepiadoideae, a highly supported clade (BS = 100%) (Fig. 1). A group of Apocynoideae consisting of *Baissea zygodioides* Stapf, *Oncinotis tenuiloba* Stapf, and *Motandra guineensis* (Thonn.) A. DC. (BS = 100%) is monophyletic and sister to the Asclepiadoideae–Secamonoideae clade (BS = 92%). Because we do not have *trnT-L* sequences for *Baissea*, *Oncinotis* Benth., and *Motandra* A. DC. (Appendix 1),

we performed a supplementary analysis based on *matK*, *trnL*, and *trnL-F* to judge if the absence of those sequences for *trnT-L* might cause a bias into our results. This supplementary analysis supported the *Baissea–Oncinotis–Motandra* clade with a BS of 88%.

Within the Secamonoideae, 13 groups are strongly supported with BS above 90% (Fig. 1). The genus *Pervillaea* with all Malagasy species included (*P. decaryi* (Choux) Klack., *P. venenata* (Baill.) Klack., *P. phillipsonii* Klack., and *P. tomentosa* Decne.) is strongly supported (BS = 97%). A fifth species of *Pervillaea* has recently been described from Mauritius (Klackenberg, 2005a). However, only 150-year-old material is known for *P. brevirostris* Klack., and it has not yet been possible to include this taxon in the analysis. The bispecific genus *Secamonopsis* is also well supported (BS = 100%), but its relationship with other genera is not resolved. Within the genus *Secamone*, several clades are well supported. This is the case for the clade including *S. cristata* Jum. & H. Perrier and *S. bosseri* Klack. (see also Fig. 2, clade 1, BS = 97%); a clade with *S. castanea*, *S. cloiselii* Choux, *S. ecoronata* Klack., *S. minutifolia* Choux, and *S. urceolata* Klack. (Fig. 2, clade 2, BS = 97%), in which *S. cloiselii*, *S. ecoronata*, *S. minutifolia*, and *S. urceolata* form a strongly supported group (BS = 93%); and a clade composed of *S. ligustrifolia* Decne. and *S. buxifolia* Decne. (Fig. 2, clade 3, BS = 100%) that lies sister to a clade including *S. glaberrima*, *S. uncinata* Choux, *S. tenuifolia* Decne., and *S. sparsiflora* Klack. (Fig. 2, clade 4, BS = 99%) in which *S. tenuifolia* and *S. sparsiflora* form a strongly supported group (BS = 100%).

The Bayesian consensus tree is highly concordant with the consensus tree based on SW (Fig. 1). It shows two more resolved nodes (one within the Periplocoideae and one within the Secamonoideae) than the parsimony consensus tree, but these nodes are not well supported. Support values (posterior probabilities, hereafter PP) for clades in the Bayesian tree, however, are generally higher than those based on parsimony. The number of clades highly supported (PP > 95%) within the Secamonoideae is 17 in the Bayesian phylogeny instead of 14 (BS > 85%) in the parsimony phylogeny (Fig. 1). Like the parsimony phylogeny, the Bayesian tree also identifies two groups of species within the principal genus *Secamone* (PP = 100%): the first including *S. oleaefolia* and *S. volubilis* (BS = 80%; PP = 100%), and the second including the other Malagasy *Secamone* (BS = 97%; PP = 100%). These two groups form a polytomy with *Secamonopsis* in both analyses. Groups of *Secamone* identified in the parsimony tree are also present in the Bayesian tree with higher PP.

Discussion

Monophyly of the Secamonoideae and their relationships within Apocynaceae

Both parsimony and Bayesian analyses of the combined four chloroplastic regions *matK*, *trnT-L*, *trnL*, and *trnL-F* support the monophyly of each of the three subfamilies of the former Asclepiadaceae (Asclepiadoideae, Periplocoideae, and Secamonoideae) with the Secamonoideae as sister to the Asclepiadoideae (Fig. 1). *Baissea zygodioides*, *Oncinotis tenuiloba*, and *Motandra guineensis* (Apocynoideae) form a monophyletic sister group to the clade constituted of Asclepiadoideae and Secamonoideae, which renders the former Asclepiadaceae s.l. (i.e., with Periplocoideae included) non-monophyletic. The monophyly of the Asclepiadaceae s. str. has been questioned by addition of derived Apocynoideae such as *Prestonia* R. Br., *Baissea*, and *Motandra* in phylogenetic studies (Sennblad & Bremer, 2000, 2002; Potgieter & Albert, 2001). Although *Baissea* and *Motandra* do not show clear morphological similarities with the Asclepiadaceae, these phylogenies showed *Baissea* to be placed within the former Asclepiadaceae but with a low support value. To date, the phylogenetic studies of derived Apocynaceae s.l. have agreed on the position of Secamonoideae as sister group to the Asclepiadoideae. Our study suggests that a group of derived Apocynoideae composed of *Baissea*, *Motandra*, and *Oncinotis* comes out as sister group to the Asclepiadoideae–Secamonoideae clade. This result is consistent with that of Livshultz et al. (2007) and does not contradict the position of *Baissea* in the analyses of Potgieter and Albert (2001) and Sennblad and Bremer (2002). *Baissea*, *Motandra*, and *Oncinotis* form the subtribe Baisseinae published by De Kruif in 1983 and recognized on a tribal level by Endress (M. Endress, pers. comm.). The Baisseinae could provide a more accurate outgroup for further studies on the phylogeny of Secamonoideae.

Relationships within Secamonoideae

Within the Secamonoideae the monophylies of the two Malagasy genera *Pervillaea* and *Secamonopsis* are strongly supported, with BS of 97% and 100%, respectively, and PP of 100% for both genera (Fig. 1). The addition of *trnL* and *trnL-F* sequences increased support of the *Pervillaea* clade from 73% (Lahaye et al., 2005) to 97% but did not resolve the relationships between *Secamone* and *Secamonopsis*. In our study the genus *Pervillaea* is sister to a clade of *Secamone* and *Secamonopsis* species. This result is congruent with a preliminary morphological phylogeny of the Seca-

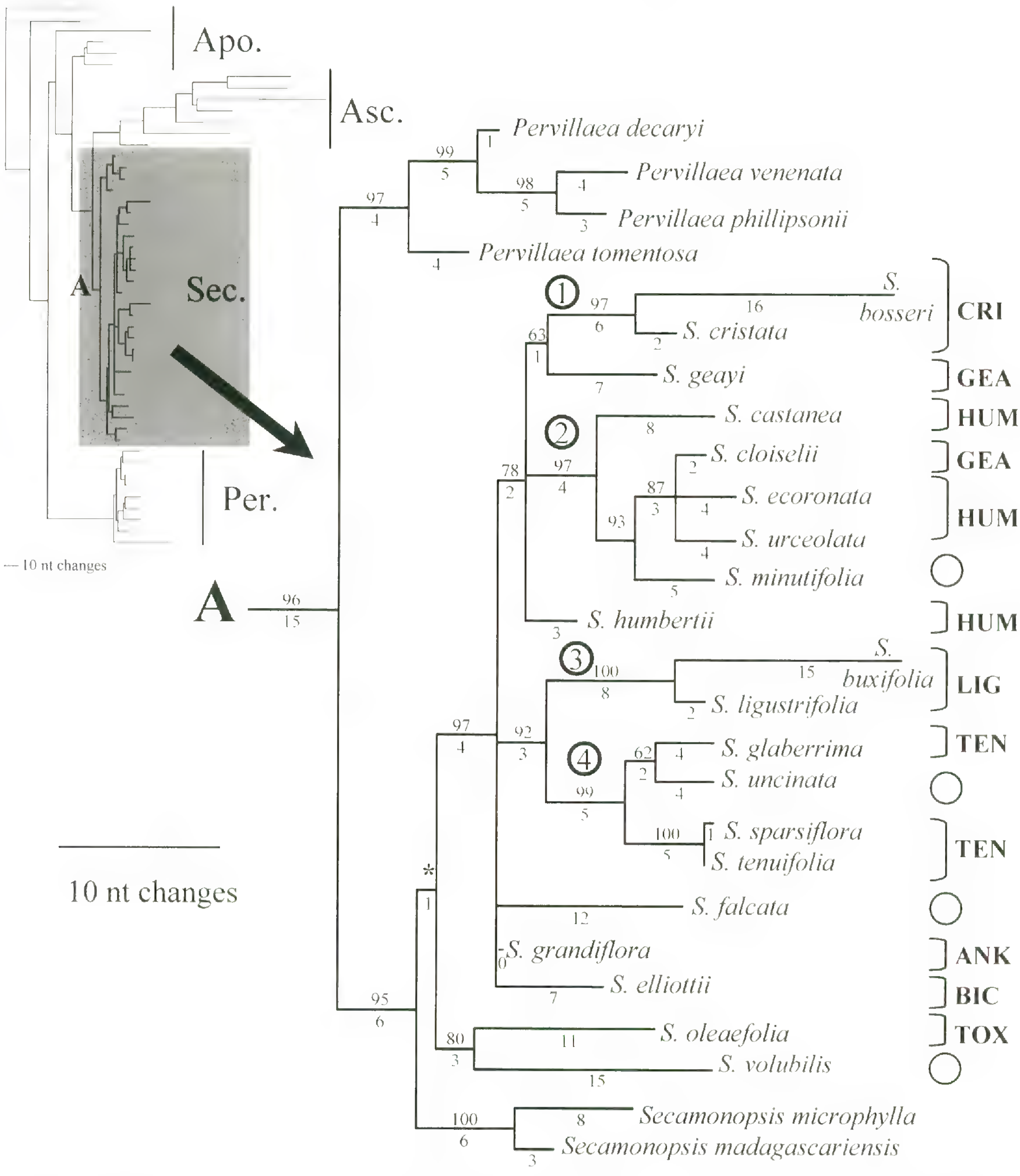

Figure 2. One of the most parsimonious trees represented as phylogram inferred from parsimony analysis (maximum parsimony + successive weighting). Bootstrap values are above branches and branch lengths below; branch scale below at the left represents 10 nucleotide (nt) changes. Morphological groups of Klackenberg (1992a) are localized on the tree. Asterisk on branch represents bootstrap value less than 50%. Abbreviations: Apo. = Apocynoideae, Asc. = Asclepiadoideae, Per. = Periplocoideae, ANK = *S. ankarensis* group, BIC = *S. bicolor* group, CRI = *S. cristata* group, GEA = *S. geayi* group, HUM = *S. humbertii* group, LIG = *S. ligustrifolia* group, TEN = *S. tenuifolia* group, TOX = *S. toxocarpoides* group, O = species not allocated to morphological group.

monoideae (Klackenberg, 1996b), which places *Pervillaea* and *Calyptranthera* as a sister group to a clade including *Genianthus*, *Toxocarpus*, *Secamone*, and *Secamonopsis*. The genus *Pervillaea* is distributed in the northern, western, and southern parts of Madagascar but is not known from the eastern part or from the central high plateau of Madagascar (Klackenberg, 1996b). *Pervillaea tomentosa* appears to be found in both the humid and dry deciduous forests of western Madagascar and has a larger ecological amplitude compared to the three other species, which are mainly restricted to the drier evergreen southern zones. The morphology-based phylogeny of *Pervillaea* (Klackenberg, 1996b) showed

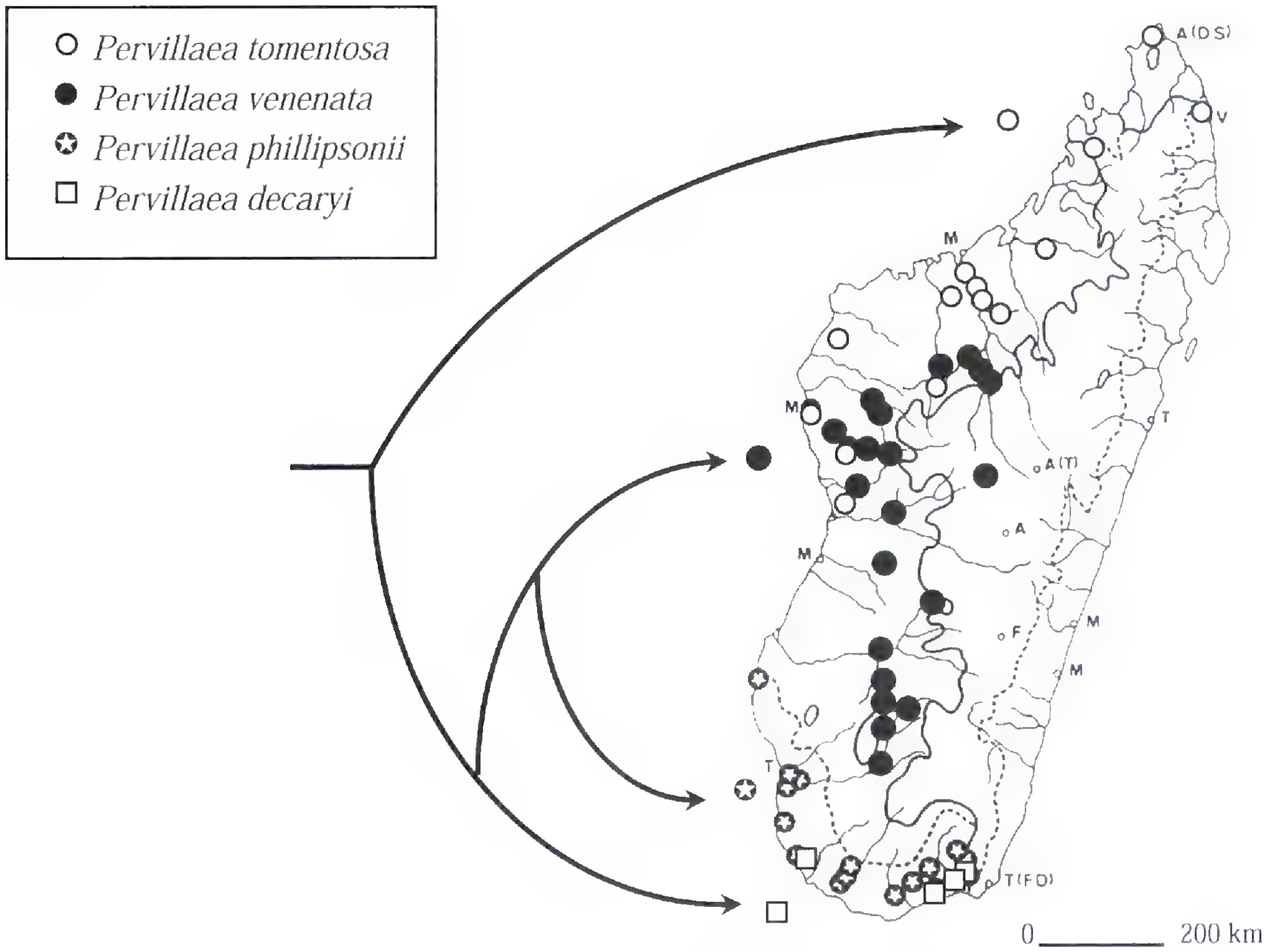

Figure 3. Schematic representation of vicariance pattern occurring within *Pervillaea* in Madagascar. Species distribution from Klackenberg (1996b).

a pattern of parallel vicariance events between western and southern phytogeographical domains in Madagascar, whereas our molecular tree suggests subsequent vicariant events (Fig. 3).

The bispecific genus *Secamonopsis* is monophyletic, but its relationship with the main genus *Secamone* remains unclear (Fig. 1). This result follows previous molecular (Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Lahaye et al., 2005) and morphological (Civeyrel & Rowe, 2001; Klackenberg, 1996b) studies. The position of *Secamonopsis* in a polytomy with one clade including *S. oleaefolia* and *S. volubilis* and a second clade consisting of the remaining *Secamone* exemplars, as shown in both the parsimony and Bayesian trees of the present study (Fig. 1), as well as in earlier studies (Civeyrel & Rowe, 2001; Lahaye et al., 2005), makes the monophyly of *Secamone* uncertain. *Secamone oleaefolia* and *S. volubilis* form a well-supported clade, but these two species do not have clear morphological or biogeographical similarities (Klackenberg, 1992a). The first is distributed on the central plateau of Madagascar, and the second is found on the island of La Réunion. *Secamone volubilis* does not share closer similarities with other *Secamone*, except with *S. salicifolia* Klack. from Mauritius, which has the same flower structure but differs in leaf shape and leaf pubescence. In contrast, *S. oleaefolia* is, on morphological grounds, thought to be related to *S. obovata* Decne. from the Malagasy eastern coastal forest and to the four species of the *S. toxocarpoides* Choux group from the Malagasy central plateau and northeastern part of the island (Klackenberg, 1992a, 2000a). They all share the characters of having long and narrow corolla lobes, long style-heads, thin-walled linear follicles, and subcoriaceous glossy leaves when dry. Species belonging to this group, including also the Socotran *S. socotrana* Balf. f., are morphologically similar to the genus *Toxocarpus* by, for example, their large flowers (usually ca. 1 cm long) and long style-head (2–3 mm long) (Klackenberg, 1992a). The addition of two new chloroplast sequences in this study did not resolve the polytomy observed between *Secamonopsis*, the *S. volubilis*–*S. oleaefolia* clade, and a clade of the remaining Malagasy species of *Secamone*.

RELATIONSHIPS WITHIN MALAGASY *SECAMONE*

Morphological (Klackenberg, 1992a) and palynological studies on the genus *Secamone* (Civeyrel, 1994; Civeyrel & Rowe, 2001) have identified characters describing groups of species within the genus. Based on both floral and vegetative characters,

Klackenberg (1992a) discussed several morphological groups within Malagasy *Secamone*, of which eight are applicable to the taxa analyzed in the present article and are mapped on Figure 2: *S. ankarensis* (Jum. & H. Perrier) Klack. group, *S. bicolor* Decne. group, *S. cristata* group, *S. geayi* Costantin & Gallaud group, *S. humbertii* Choux group, *S. ligustrifolia* group, *S. tenuifolia* group, and *S. toxocarpoides* group. Groups of *Secamone* have also been described from palynological studies, which identified six types of pollinaria (Civeyrel, 1994). The present molecular phylogeny allows us to evaluate the phylogenetic value of the morphological and palynological characters in regard to four representative clades including one or two morphological groups (Fig. 2).

Clade 1. *Secamone bosseri* and *S. cristata* of this clade (Fig. 2), representing the *S. cristata* morphological group of three species and three subspecies, are characterized by having small flowers (only 2–2.5 mm long) with long corolla tubes (tubes distinctly longer than the lobes) and usually dense inflorescences (Klackenberg, 1992a). They constitute a strongly supported clade (PP = 100%, Fig. 1). The Bayesian analysis shows that *S. geayi* forms a well-supported clade (PP = 93%) with these two species, but this is not the case after successive weighting (BS = 63%). *Secamone geayi*, *S. cristata*, and *S. bosseri* share the same type of compact pollinarium (Civeyrel, 1994). *Secamone geayi* and *S. bosseri* are restricted to the dry southwestern part of Madagascar, whereas the range of the *S. cristata* subspecies also extends as far as the northwest of the island. The three species are sympatric in southwestern Madagascar.

Clade 2. This clade (BS = 97%) is formed by four species from two different morphological groups and one species that does not fit into any group (Klackenberg, 1992a): *Secamone castanea*, *S. ecoronata*, *S. urceolata* (*S. humbertii* group), *S. cloiselii* (*S. geayi* group), and *S. minutifolia* (unidentified group). The *S. humbertii* group of Klackenberg (1992a, 1997b) consists of 10 species, of which four are included in the present study, viz., *S. castanea*, *S. ecoronata*, *S. urceolata*, as well as *S. humbertii*, which is placed in a polytomy just outside clade 2. This species group is characterized by having pouch-like structures on the stamens, but this character is absent in *S. cloiselii* and *S. minutifolia*. *Secamone cloiselii* was thought to be morphologically more similar to *S. geayi* (Klackenberg, 1992a) due to a kind of small leaf hair that is unique in *Secamone*. In the present analysis, however, these two species are placed in two different clades. *Secamone minutifolia*, with its characteristic equifacial leaves, has not been assigned to a morphological group and does not show clear similarities with the other species (Klackenberg, 1992a). Species composing clade 2 share with the two exemplars from the *S. cristata* group (clade 1) a common type of compact pollinarium (Civeyrel, unpublished). These species form a well-supported (BS = 78%; PP = 100%) clade, suggesting that the compact pollinarium with the four pollinia supported by a narrow corpuscle and a short caudicle without a distinct shape is of good phylogenetic value for this group of *Secamone*. The species of this clade are found in diverse phytogeographical areas across Madagascar.

Clade 3. This strongly supported clade (BS = 100%) includes *Secamone ligustrifolia* and *S. buxifolia*. Together with two more species not analyzed here they form the *S. ligustrifolia* morphological species group, which is characterized by large and fleshy coronal lobes with rounded back and a style-head with a distinctive lower and broader part (Klackenberg, 1992a, 2000a). *Secamone ligustrifolia* and *S. buxifolia* are distributed sympatrically in central Madagascar. They have the same type of pollinarium (Civeyrel, unpublished), with an elongated caudicle bearing four almost sessile pollinia on the back.

Clade 4. Four species, *Secamone glaberrima*, *S. uncinata*, *S. tenuifolia*, and *S. sparsiflora* form this strongly supported clade (BS = 99%). The *S. tenuifolia* group, with eight species (Klackenberg, 1992a, 2000b) distributed mostly in central but also in eastern Madagascar, is characterized by having thick corolla lobes finely puberulous on their inner side and well-developed corona lobes narrowed and puberulous in their basal part. *Secamone uncinata* from southwestern Madagascar does not share these characters, but the flowers are valvate as in the *S. tenuifolia* group, in contrast to the more general state of overlapping corolla lobes that is characteristic for all other *Secamone* included in this study, as well as for *Pervillaea* and *Secamonopsis*. Furthermore, the species in clade 4 show clear similarities in their pollinaria, which have no synorganization between pollinia, but this character is believed to be plesiomorphic (Civeyrel & Rowe, 2001).

Secamone elliottii K. Schum. is the only species out of the nine belonging to the *S. bicolor* group (Klackenberg, 1992a, 2005b) that has been included in the present analysis. This could explain its

unresolved position within the Malagasy *Secamone*. The *S. bicolor* group is characterized by entirely glabrous corollas, minute staminal columns with thin corona lobes, and tuberculate-papillate leaf epidermis. The positions of *S. falcata* Klack. and *S. grandiflora* Klack. are also unresolved. *Secamone grandiflora* belongs together with four more species to the *S. ankarensis* group of Klackenberg (1992a, 2000c). They are characterized by large flowers (ca. 1 cm long), distinctive falcate corona lobes, and a distinctly protruding and deeply bifid style-head. Morphologically, *S. falcata* adheres to this group but differs by its shorter style-head and smaller leaves on brachyblasts.

PHYLOGENETIC VALUE OF THE INDELS RELATED TO THE SECAMONOIDEAE

In the coding gene *matK*, the most informative indels (Appendix 2) are the five bp deletion (sites 81 to 85, Appendix 2) and the four bp deletion (sites 92 to 95), both found in a previous study (Civeyrel & Rowe, 2001) for the clade of Malagasy *Secamone*. These deletions are not found in *S. volubilis* distributed in La Réunion, nor in *S. oleaefolia* from central Madagascar. These two species also show an insertion of six bp in the *matK* gene from sites 75 to 80.

In the non-coding region *trnT-L*, seven indels are phylogenetically informative for the Secamonoideae. The entire Secamonoideae can be characterized by an insertion of 10 bp localized from sites 1833 to 1842 (Appendix 2). The clade including *Secamone* and *Secamonopsis* can be characterized by an insertion of seven bp from sites 1918 to 1924. The two species of the genus *Secamonopsis* can be characterized by a deletion of seven bp from sites 1782 to 1788. The clade of Malagasy *Secamone* (excluding *S. oleaefolia*) shows an insertion of seven bp from sites 1911 to 1917. Within this clade, a group including *S. cloiselii*, *S. ecoronata*, and *S. urceolata* (clade 2, Fig. 2), is characterized by an insertion of five bp from sites 1620 to 1624. The *Secamone volubilis–S. oleaefolia* clade shows an insertion of two bp from sites 1739 to 1740, and a deletion of one nucleotide at the position 1994.

The interestingly conservative *trnL* intron does not show unambiguous informative indels for the Secamonoideae. The *trnL-F* spacer shows two informative indels: an insertion of one nucleotide at position 3214 shared by the entire Secamonoideae, and a deletion of one nucleotide at the site 3380 shared by all analyzed species of *Pervillaea*. Each major taxonomic group within the Secamonoideae shows at least one characteristic indel. Regarding the phylogenetic value of the indels within the subfamily, most noteworthy is the high number of indels characterizing the *Secamone volubilis–S. oleaefolia* clade, which are not present in the remaining Malagasy *Secamone*. However, this molecular evidence is not incongruent with the current generic boundaries in Secamonoideae. Consequently, based on the present sampling, it seems premature to redraw generic circumscriptions, as *S. volubilis* and *S. oleaefolia* both fit well in *Secamone* morphologically. On the other hand, *Secamonopsis*, which is morphologically distinct with its characteristic two-armed caudicles, is at present best kept as a separate genus.

ISLAND COLONIZATION AND DIVERSIFICATION: THE RADIATION OF THE SECAMONOIDEAE IN MADAGASCAR

Sequence divergence calculated among the Malagasy *Secamone* species ranged from 0.03% to 1.6%, and for most of the species branch lengths do not exceed 10 (Fig. 2) on a total tree length of 1153. This low sequence variation is concomitant with an important morphological diversification, namely, the morphology of staminal coronas and style-heads that characterizes different groups of *Secamone* (Klackenberg, 1992a). This low sequence divergence coupled with an important morphological differentiation (here the breeding system) is consistent with other molecular studies related to adaptive radiation of taxa in islands or other specific areas, such as high African mountains (Knox & Palmer, 1995; Böhle et al., 1996; Kim et al., 1996; Baldwin et al., 1998; Panero et al., 1999; Moore et al., 2002; Mort et al., 2002; Bruyns & Klak, 2004). The Secamonoideae have colonized all of the phytogeographical domains in Madagascar with a high percentage of endemism, with around 80% of the species endemic to one domain only (Klackenberg, 1992a). Sympatric distributions of species are not rare. For example, *S. sparsiflora* and *S. minutifolia*, belonging to two different groups within *Secamone* but very similar in habit, are both endemic to the Isalo plateau and can be found within a few meters of each other. The important radiation and speciation of the Secamonoideae observed in Madagascar could be attributed to successive geographic vicariance events across different ecological zones. To test this hypothesis, it is necessary to get a more complete phylogeny of the Malagasy *Secamone*.

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Appendix 1. Taxa used in the molecular phylogeny of Secamonoideae. Accession numbers for sequences in GenBank are provided. *matK* (maturase-encoding gene), *trnT-L* (spacer), *trnL* (intron), and *trnL-F* (spacer) are sequences in the chloroplast genome. New sequences for this study are represented by an asterisk; unavailable sequences are represented by a dash.

Taxon	Taxon voucher, herbarium, origin	GenBank accession number			
		matK	*trnT-L*	*trnL*	*trnL-F*
OUTGROUP					
Rauvolfioideae					
Acokanthera oblongifolia (Hochst.) Codd	*Civeyrel 1053*, TL, South Africa	Z70182	AY899957	DQ221123*	DQ221165*
Apocynoideae					
Beaumontia grandiflora Wall.	*Civeyrel 1071*, TL, India	Z98174	AY899958	DQ221124*	DQ221166*
Nerium oleander L.	*Civeyrel 1079*, TL, Europe	Z98173	AY899959	DQ221125*	DQ221167*
INGROUP					
Apocynoideae					
Baissea zygodioides Stapf	*P. Endress 99-02*, Z, Ivory Coast	DQ221120*	—	DQ221126*	DQ221168*
Motandra guineensis (Thonn.) A. DC.	*Pascal Markin s.n.*, Z, Ivory Coast	DQ221121*	—	DQ221127*	DQ221169*
Oncinotis tenuiloba Stapf	*Abbot 7707*, Z, South Africa	DQ221122*	—	DQ221128*	DQ221170*
Asclepiadoideae					
Araujia sericifera Brot.	*Civeyrel 1059*, TL, South America	Z98194	AY899960	DQ221129*	DQ221171*
Cynanchum acutum L.	*Civeyrel 1644*, TL, France (cultivated)	AY899939	AJ428552	AJ428583	AJ428584
Fockea capensis Endl.	*Civeyrel 1067*, TL, South Africa	Z98187	AY899961	DQ221131*	DQ221173*
Gonolobus xanthotrichus Brandegee	*Civeyrel 1060*, TL, Mexico	Z98195	AY899962	DQ221133*	DQ221175*
Pergularia daemia Chiov.	*Civeyrel 1000*, TL, India	Z98191	AJ290891	AJ290892	AJ290893
Riocreuxia burchellii Schum.	*Civeyrel 1109*, TL, Africa	Z98190	AY899963	DQ221143*	DQ221185*
Vincetoxicum nigrum (L.) Moench	*Civeyrel 1106*, TL, Europe	Z98192	AY899964	AF214451	AF214297
Periplocoideae					
Camptocarpus mauritianus Decne.	*Civeyrel 1062*, TL, Réunion Island	Z98175	AY899965	DQ221130*	DQ221172*
Ischnolepis graminifolia (Costantin & Gallaud) Klack.	*Civeyrel 1246*, TL, Madagascar	AY899940	AY899966	DQ221134*	DQ221176*
Mondia ecornuta (N. E. Br.) Bullock	*Sennblad 215*, K, Africa	AY899941	AY899967	DQ221135*	DQ221177*
Pentopetia grevei (Baill.) Venter	*Civeyrel 1222*, TL, Madagascar	AY899943	AY899968	DQ221132*	DQ221174*
Pentopetia lutea Klack. & L. Civeyrel	*Civeyrel, 1243b*, TL, Madagascar	AY899944	AY899969	DQ221136*	DQ221178*
Periploca graeca L.	*Civeyrel 1083*, TL, Greece	Z98178	AY899970	AF102468	AF214244
Petopentia natalensis (Schltr.) Bullock	*Civeyrel 1173*, TL, France (cultivated)	AY903453	AJ581810	DQ221141*	DQ221183*
Raphionacme welwitschii Schltr. & Rendle	*Civeyrel 1088*, TL, Zaire	Z98179	AY899971	DQ221142*	DQ221184*
Tacazzea apiculata Oliv.	*Venter 9188*, K, Africa	AY899945	AY899972	DQ221164*	DQ221206*

Appendix 1. Continued.

Taxon	Taxon voucher, herbarium, origin	GenBank accession number			
		matK	*trnT-L*	*trnL*	*trnL-F*
Secamonoideae					
Pervillaea decaryi (Choux) Klack.	*Civeyrel 3471*, TL, Madagascar	AY899946	AY899973	DQ221137*	DQ221179*
Pervillaea phillipsonii Klack.	*Civeyrel 1241*, TL, Madagascar	AJ312408	AY899976	DQ221140*	DQ221182*
Pervillaea tomentosa Decne.	*Civeyrel 1522*, TL, Madagascar	AY899947	AY899975	DQ221139*	DQ221181*
Pervillaea venenata (Baill.) Klack.	*Civeyrel 1248*, TL, Madagascar	Z98181	AY899974	DQ221138*	DQ221180*
Secamone bosseri Klack	*Civeyrel 1267*, TL, Madagascar	Z98182	AY899977	DQ221144*	DQ221186*
Secamone buxifolia Decne.	*Civeyrel 1322*, TL, Madagascar	AJ312405	AY899978	DQ221145*	DQ221187*
Secamone castanea Klack.	*Civeyrel 1356*, TL, Madagascar	AY899948	AY899979	DQ221146*	DQ221188*
Secamone cloiselii Choux	*Civeyrel 1312*, TL, Madagascar	AY899949	AY899980	DQ221147*	DQ221189*
Secamone cristata Jum. & H. Perrier	*Civeyrel 1320*, TL, Madagascar	AJ312400	AY899981	DQ221148*	DQ221190*
Secamone ecoronata Klack.	*Civeyrel 1261*, TL, Madagascar	AJ312407	AY899982	DQ221149*	DQ221191*
Secamone elliottii K. Schum.	*Civeyrel 1304*, TL, Madagascar	AJ312402	AY899983	DQ221150*	DQ221192*
Secamone falcata Klack.	*Civeyrel 1228*, TL, Madagascar	AJ312404	AY899984	DQ221151*	DQ221193*
Secamone geayi Costantin & Gallaud	*Civeyrel 1200*, TL, Madagascar	Z98184	AY899985	DQ221152*	DQ221194*
Secamone glaberrima K. Schum.	*Civeyrel 1335*, TL, Madagascar	AY899950	AY899986	AF214420	AF214266
Secamone grandiflora Klack.	*Civeyrel 1292*, TL, Madagascar	AY899951	AY899987	DQ221153*	DQ221195*
Secamone humbertii Choux	*Civeyrel 1293*, TL, Madagascar	AY899952	AY899988	DQ221154*	DQ221196*
Secamone ligustrifolia Decne.	*Civeyrel 1323b*, TL, Madagascar	AY899953	AY899989	DQ221155*	DQ221197*
Secamone minutifolia Choux	*Civeyrel 1257*, TL, Madagascar	AJ312406	AY899990	DQ221156*	DQ221198*
Secamone oleaefolia Decne.	*Civeyrel 1770*, TL, Madagascar	AY899954	AY899991	AF214421	AF214267
Secamone sparsiflora Klack.	*Civeyrel 1244*, TL, Madagascar	AJ312401	AY899992	DQ221157*	DQ221199*
Secamone tenuifolia Decne.	*Civeyrel 1259*, TL, Madagascar	AY899955	AY899993	DQ221158*	DQ221200*
Secamone uncinata Choux	*Civeyrel 1309*, TL, Madagascar	AJ312403	AY899994	DQ221160*	DQ221202*
Secamone urceolata Klack.	*Civeyrel 1299*, TL, Madagascar	AY899956	AY899995	DQ221159*	DQ221201*
Secamone volubilis (Lam.) Marais	*Civeyrel 1092*, TL, Réunion Island	Z98186	AY899996	DQ221161*	DQ221203*
Secamonopsis madagascariensis Jum.	*Civeyrel 1262*, TL, Madagascar	Z98185	AY899998	DQ221163*	DQ221205*
Secamonopsis microphylla L. Civeyrel & Klack.	*Civeyrel 1206*, TL, Madagascar	AJ312409	AY899997	DQ221162*	DQ221204*

Appendix 2. Informative indels related to the Secamonoideae. ? represents base not available.

	MatK		*trnT-L*						*trnL-F*	
	777777888888	9999	1111111 6666666 1222222	1111 7777 3344	111111111 777777777 888888888	11111111111 88888888888 33333333444	1111111111111111 9999999999999999 1111111111222222	111 999 999	3 2 1	3 3 8
	456789012345	2345	9012345	8901	123456789	23456789012	0123456789012345	345	4	0
Acokanthera oblongifolia	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	G----------	T--------------A	TAA	-	T
Beaumontia grandiflora	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATAAT---	T--------------C	TAA	-	T
Nerium oleander	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	G----------	T--------------C	TAA	-	T
Baissea zygodioides	T------AGTTT	AAAC	???????	????	?????????	??????????	????????????????	???	-	T
Motandra guineensis	T------AGTTT	AAAC	???????	????	?????????	??????????	????????????????	???	-	T
Oncinotis tenuiloba	T------AGTTT	AAAC	???????	????	?????????	??????????	????????????????	???	-	T
Araujia sericifera	T------AGTTT	AAAC	A-----T	A--G	TAGAATTTA	G----------	T--------------C	TAA	-	T
Cynanchum acutum	T------AGTTT	AAAC	A-----T	----	---------	----------	----------------	TAA	-	T
Fockea capensis	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	G----------	T--------------C	TAA	-	T
Gonolobus xanthotrichus	T------AGTTT	AAAC	A-----T	A--G	TAGAATTTA	G----------	T--------------C	TAA	-	G
Pergularia daemia	T------AGTTT	AAAC	A-----T	A--G	TAGAATTTA	G----------	T--------------C	TAA	-	T
Riocreuxia burchellii	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	G----------	T--------------C	TAA	-	T
Vincetoxicum nigrum	T------AGTTT	AAAC	A-----T	A--G	TAGAATTTA	G----------	T--------------C	TAA	-	T
Camptocarpus mauritianus	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T--------------C	TAA	-	T
Ischnolepis graminifolia	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T--------------C	TAA	-	T
Mondia ecornuta	T------AGTTT	AAAC	A-----A	A--G	TACAATTTA	GATATCTT---	T--------------C	TAA	-	T
Pentopetia grevei	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T--------------C	TAA	-	T
Pentopetia lutea	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T--------------C	TAA	-	?
Periploca graeca	T------AGTTT	AAAC	A-----T	A--G	TACAATTTC	GATATCTTATA	T--------------C	TAA	-	T
Petopentia natalensis	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T--------------C	TAA	-	T
Raphionacme welwitschii	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T--------------C	TAA	-	T
Tacazzea apiculata	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T--------------C	TAA	-	?
Pervillaea decaryi	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATTTATTACG	T--------------C	TAA	T	-
Pervillaea venenata	T------AGTTT	AAAC	A-----T	A--A	TACAATTTA	GATTTATTACG	T--------------C	TAA	T	-
Pervillaea tomentosa	T------AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATATTACG	T--------------C	TAA	T	-
Pervillaea phillipsonii	T------AGTTT	AAAC	A-----T	A--A	TACAATTTA	GATTTATTACG	T--------------C	TAA	T	-
Secamone bosseri	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone buxifolia	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone castanea	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone cloiselii	T----------	----	ATGTTAT	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T

Appendix 2. Continued.

	MatK		*trnT-L*						*trnL-F*	
	777777888888	9999	1111111 6666666 1222222	1111 7777 3344	111111111 777777777 888888888	11111111111 88888888888 33333333444	1111111111111111 9999999999999999 1111111111222222	111 999 999	3 2 1	3 3 8
	456789012345	2345	9012345	8901	123456789	23456789012	0123456789012345	345	4	0
Secamone cristata	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone ecoronata	T----------	----	ATGTTAT	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone elliottii	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone falcata	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone geayi	T----------	----	A-----T	A--G	TACAATTTA	GATATTTTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone glaberrima	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	T???????????????	???	T	T
Secamone grandiflora	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone humbertii	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone ligustrifolia	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone minutifolia	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone sparsiflora	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone tenuifolia	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone urceolata	T----------	----	ATGTTAT	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone uncinata	T----------	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTCGAGATTC	TAA	T	T
Secamone oleaefolia	TGACCATGGTTT	AAAC	A-----T	AGCG	TACAATTTA	GATATATTACG	T-------CGAGATTC	T-A	T	T
Secamone volubilis	TGACCATGGTTT	AAAC	A-----T	AGCG	TACAATTTA	GATATATTACG	T-------CGAGATTC	T-A	T	T
Secamonopsis microphylla	T------GGTTT	AAAC	A-----T	A--G	T-------A	GATATATTACG	T-------CGAGATTC	TAA	T	G
Secamonopsis madagascariensis	T------GGTTT	AAAC	A-----T	A--G	T-------A	GATATATTACG	T-------CGAGATTC	TAA	T	T

CEROPEGIA (APOCYNACEAE, CEROPEGIEAE, STAPELIINAE): PARAPHYLETIC BUT STILL TAXONOMICALLY SOUND[1]

Ulrich Meve[2] *and Sigrid Liede-Schumann*[2]

ABSTRACT

Even though the species-rich genus *Ceropegia* L. (Apocynaceae, Ceropegieae) is convincingly characterized by its pitfall flowers, investigation of non-coding markers of chloroplast DNA (cpDNA) (*trnT-L* and *trnL-F* spacers and the *trnL* intron) and nuclear ribosomal DNA (nrDNA) (ITS) has shown that *Ceropegia* is twice paraphyletic. The 36 analyzed *Ceropegia* taxa scatter over a grade of seven clades. One clade is shared by *Ceropegia* and all *Brachystelma* R. Br. species investigated, making *Ceropegia* (without *Brachystelma*) paraphyletic. All endemic Madagascan *Ceropegia* taxa investigated and the East African *C. robynsiana* Werderm. share a terminal, but not further-resolved clade with the stapeliads. Thus, again, *Ceropegia* without the stapeliads is paraphyletic. These results are incongruent with current taxonomy. In the absence of adequate morphological, anatomical, or karyological characters supporting a taxonomic reclassification of the genus in accordance with the retrieved clades of the phylogenetic analysis, it is proposed that the current taxonomy be maintained.

Key words: *Brachystelma*, *Ceropegia*, ITS, molecular systematics, morphology, paraphyly, phylogeny, *trnL* intron, *trnT-L* and *trnL-F* spacers.

With more than 180 species, *Ceropegia* L. (Apocynaceae, Asclepiadoideae) represents the largest genus in the Old World tribe Ceropegieae, where it belongs to the subtribe Stapeliinae G. Don together with *Brachystelma* R. Br. and the 40 stapeliad genera, which form a statistically strongly supported clade (100% bootstrap percentage [BP]) in previous cladistic analyses of chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) sequence data (cf. Meve & Liede, 2002a, 2004). The distribution of *Ceropegia* extends across the tribal range from West Africa to Australia, with two main centers of distribution: East Africa including southeastern Africa (115 species) and India (40 species). Additional subcenters are found in Madagascar (20 species) and China (17 species). The fascinating forms and function of the *Ceropegia* lantern and pitfall flowers have long attracted the attention of biologists dealing with aspects of both flower morphology and pollination biology (e.g., Vogel, 1961, 1962), as well as horticulturists, because *C. linearis* E. Mey. subsp. *woodii* (Schltr.) H. Huber is an important ornamental. Also, the extreme variability of flower morphology in the genus and within many specific and infraspecific taxa has been well utilized by taxonomists. Over 500 different taxon names have been described, of which an increasing number have been sunk into synonymy (Meve & Mangelsdorff, 2001; Meve et al., 2001; Meve, 2002). The current concept of relatively few but variable species is increasingly supported by molecular data demonstrating low genetic divergence, even between morphologically rather different-looking variants, as in the *C. aristolochioides* complex (Meve et al., 2001). In this random amplified polymorphic DNA (RAPD) analysis, estimates of genetic distances were low (0.150) to very low (0.008). Apart from regional taxonomic treatments (Dyer, 1983; Ansari, 1984; Meve & Liede, 1994a; Masinde, 2000; Gilbert, 2003), only one full revision of *Ceropegia* (151 species; Huber, 1957) and an almost complete treatment (160 species, omitting only the ca. 25 non-succulent species; Meve, 2002) are available. Infrageneric classifications were proposed by both Schumann (1895) and Huber (1957). Schumann (1895) created three sections on the basis of the degree of corolla fusion and orientation of the lobes. Huber (1957) discarded Schumann's (1895) concept, assigning species to 20 sections (and 23 series). Meve and Liede (1994a) additionally described another section, *Ceropegia* sect. *Dimorpha* Huber ex Meve & Liede, for the stem-succulent taxa endemic to Madagascar. Huber (1957) treated *Riocreuxia* Decne. as a section of *Ceropegia*, but Dyer (1980) correctly reinstated the genus, as demonstrated morphologically

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(Meve, 1998) and later molecularly (Meve & Liede, 2002a, 2004). The pitfall flowers of *Riocreuxia* are now regarded as a case of homoiology (parallel evolution; Meve & Liede, 1999, 2004). As criticized by Dyer (1980) and Meve and Liede (1994a), Huber's subdivison of the genus, which is based nearly exclusively on flower morphological characters, is highly artificial. To date, no phylogenetic analysis of the genus *Ceropegia* as a whole is available. Considering the increased awareness of the extreme variability and the many parallelisms, both vegetative and floral, in *Ceropegia* (and Ceropegieae), an analysis of the infrageneric phylogeny of *Ceropegia* is overdue. In the present paper we focus on four main goals: (1) reconstruction of the phylogeny of *Ceropegia* using cpDNA and nrDNA sequence data and comparison with root and flower morphology; (2) deduction of the geographic origin of *Ceropegia* and its colonization of Madagascar; (3) reconsideration of the status of *Brachystelma*; and (4) discussion on how to translate our phylogeny into a reasonable taxonomy and how to deal with paraphyletic taxa (cf. Brummitt, 1996, 2002; Grant, 2003).

Material and Methods

TAXA SAMPLED

The material used in this study is summarized in Table 1, including 69 voucher specimens, authors of species, and donors or collectors of material. For the ingroup, 36 species of *Ceropegia* and 10 species of *Brachystelma* were chosen, covering the range of diversity and distribution of the genus. As outgroups, representatives were chosen from two other subtribes of Ceropegieae (cf. Meve & Liede, 2004), Anisotominae (*Anisotoma* Fenzl., *Neoschumannia* Schltr., *Riocreuxia*, and *Sisyranthus* E. Mey.) and Leptadeniinae (*Leptadenia* R. Br. and *Orthanthera* Wight), and from the most distant outgroup, Marsdenieae (*Gymnema sylvestre* (Retz.) Schult.; Table 1). Representatives of eight species of different stapeliad genera were selected as the closest outgroup.

CHROMOSOME NUMBERS AND CHROMOSOME LENGTH

Chromosome numbers and chromosome sizes were established from adventitious root tip squash preparations. Ten mitotic metaphase plates of each sample were investigated. The root tips were pretreated in 0.002 M hydroxyquinoline for 4 hr. at 20°C (Tjio & Levan, 1950), fixed in Carnoy's solution for 24 hr. at 20°C, and stained in carmine for 24 hr. at 60°C (Snow, 1963). For cytological investigation, a Leitz Orthoplan microscope (Leica Microscopic Systems, Wetzlar, Germany) was used. The total length of all chromosomes in the genome was measured on maximally contracted chromosomes on the basis of camera lucida pencil drawings.

DNA EXTRACTION AND PCR

DNA was isolated from fresh leaf and stem tip tissue according to Doyle and Doyle (1987). Polymerase chain reaction (PCR) primers and protocol for the plastid *trnT-L* and *trnL-F* spacers and the *trnL* intron correspond to Taberlet et al. (1991). Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer (Applied Biosystems, Foster City, California). One hundred seventeen cpDNA sequences and 32 ITS sequences were used from previous studies of the authors (cf. Meve & Liede, 2001, 2004), and 99 cpDNA and 33 ITS sequences were newly obtained for this study (Table 1). No ITS sequences could be generated for *Brachystelma franksiae* N. E. Br., *B. schizoglossoides* N. E. Br., and *Ceropegia maccannii* Ansari. The ITS sequence of the marsdeniad *Gymnema sylvestre* could not be aligned with the ITS sequences of the Ceropegieae taxa and was therefore omitted. All sequences have been deposited at the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database (for EMBL accession numbers, see Table 1).

DNA DATA ANALYSIS

Sequences were pre-aligned with Sequence Navigator Version 1.0.1 (Perkin-Elmer, Inc., Waltham, Massachusetts); the alignment was cleaned manually. Indels were coded as missing throughout. Phylogenetic analysis and tests for clade support were performed using PAUP version 4.0b10 (PPC) (Swofford, 1998). Phylogenies were generated using Fitch parsimony as implemented in PAUP* employing heuristic searches, with 1000 replicates, random stepwise addition, MULPARS off, and tree bisection-reconnection (TBR) branch swapping. The resulting trees were then used as starting trees for a second round of searches with MULPARS on. Our search strategy was aimed at finding as many different islands of trees as possible. Sets of equally parsimonious trees recovered from each analysis were summarized by strict consensus. BPs (Felsenstein, 1985) derived from 1000 replicates (saving a maximum of 100 trees per replicate) were calculated as measures of support for individual clades. To test whether cpDNA data and ITS data can be combined, a partition homogeneity test, as implemented in PAUP*, was conducted.

Table 1. Voucher and locality information and EMBL accession numbers for plant material used in the molecular studies (newly published sequences are marked by *).

Species	Origin	Voucher	EMBL acc. no. *trnT-L* spacer *trnL* intron *trnL-F* spacer	EMBL acc. no. ITS
Outgroup				
Gymnema sylvestre (Retz.) Schult. (Marsdenieae)	Cameroon: E Mokolo	*Meve 919* (B, UBT)	AJ402118 AJ402137 AJ402142	—
Anisotoma cordifolia Fenzl	South Africa: Eastern Cape	*Nicholas 2811* (UDW)	AJ410016 AJ410017 AJ410018	AJ310780
Apteranthes europaea (Guss.) Plowes var. *europaea*	Spain: Capo da Gata	*Albers 87-23-004-20* (MSUN)	AJ488312 AJ488313 AJ488314	AJ488773
Boucerosia frerei (G. D. Rowley) Meve & Liede	India: Maharashtra	*Sakaria* sub *K 781* (MSUN)	AJ488324 AJ488325 AJ488326	AJ488777
Caralluma arachnoidea (P. R. O. Bally) M. G. Gilbert	Kenya: Rukanga	*Meve 934* (UBT)	AJ410037 AJ410038 AJ410039	AJ310785
Caudanthera edulis (Edgew.) Meve & Liede	Oman: W Salalah	*Butler C312* (UBT)	AJ402116 AJ402139 AJ402140	AJ402162
Desmidorchis flava (N. E. Br.) Meve & Liede	Oman: Mugsail	*Collenette s.n.* (MSUN)	AJ488357 AJ488358 AJ488359	AJ488789
Echidnopsis repens R. A. Dyer & Verdoorn	Kenya: Mt. Maktau	*Meve 942* (MSUN, UBT)	AJ488381 AJ488382 AJ488383	AJ488797
Hoodia officinalis (N. E. Br.) Plowes	Namibia: E Aus	*Meve 176* (UBT)	AJ488393 AJ488394 AJ488395	AJ488801
Larryleachia cactiformis (Hook.) Plowes	South Africa: Numees	*Teissier 097* (UBT)	AJ402120 AJ402135 AJ402144	AJ402159
Leptadenia arborea (Forssk.) Schweinf.	Egypt: Asswan	*Heneidak s.n.* (Suez Canal Univ. Herb.)	AJ574832 AJ574833 AJ574834	AM493305*
Leptadenia hastata Decne.	Gambia: Abouko NP	*S. Huber s.n.* (UBT)	AJ410055 AJ410056 AJ410057	AJ310787
Neoschumannia cardinea (S. Moore) Meve	Tanzania: Amani Forest Reserve	*Liede & Meve 3359* (B, UBT)	AJ410049 AJ410050 AJ410051	AJ310790
Neoschumannia kamerunensis Schltr.	Cameroon: Mt. Cameroon	*Meve & Etuge 910* (B, K, UBT)	AJ410052 AJ410053 AJ410054	AJ310791
Orthanthera albida Schinz	Namibia: W Marinkas Quellen	*Bruyns 7262* (BOL)	AJ410058 AJ410059 AJ410060	AJ310792
Orthanthera jasminiflora (Decne.) N. E. Br. ex Schinz	Botswana: Shakawe	*Bruyns 6491* (BOL, K)	AJ410064 AJ410065 AJ410066	AJ310794
Quaqua incarnata (L. f.) Bruyns subsp. *incarnata*	South Africa: S Klawer	*Albers & Meve 06* (MSUN)	AJ488453 AJ488454 AJ488455	AJ488821

Table 1. Continued.

Species	Origin	Voucher	EMBL acc. no. *trnT-L* spacer *trnL* intron *trnL-F* spacer	EMBL acc. no. ITS
Riocreuxia burchellii K. Schum.	South Africa: KwaZulu-Natal	*ex hort. Shirley* (MSUN)	AJ488306 AJ488307 AJ488308	AJ488771
Riocreuxia torulosa Decne.	South Africa: KwaZulu-Natal	*Edwards s.n.* (UDW)	AJ574841 AJ574842 AJ574843	AM493295*
Sisyranthus compactus N. E. Br.	South Africa: Kei Mouth	*Nicholas 2825* (UDW)	AJ410067 AJ410068 AJ410069	AJ310795
Sisyranthus trichostomus K. Schum.	South Africa: KwaZulu-Natal	*Ollerton 245* (NU)	AJ574846 AJ574845 AJ574844	AM493296*
Sisyranthus virgatus E. Mey.	South Africa: KwaZulu-Natal	*Nicholas & Hariparsaad 2855* (UDW)	AJ574847 AJ574848 AJ574849	AM493297*
Stapelia glanduliflora Mass.	South Africa: S Klawer	*Albers & Meve 04* (MSUN)	AJ402127 AJ402128 AJ402151	AJ402152
Ingroup				
Brachystelma burchellii (Decne.) Peckover	South Africa: Bloemfontein	*Peckover sub Specks 3156* (UBT)	AJ410046 AJ410047 AJ410048	AJ310789
Brachystelma christianeae Peckover	South Africa: Nkandla	*Peckover sub Specks 2540* (UBT)	AJ410070 AJ410071 AJ410072	AJ310796
Brachystelma ellipticum A. Rich. (= *B. lineare* A. Rich.)	Ethiopia: Bahir Dar	*Specks 432* (K, MSUN)	AJ410019 AJ410020 AJ410021	AJ310781
Brachystelma filifolium (Schltr.) Peckover	South Africa: White River	*Peckover sub Specks 2540* (UBT)	AJ410073 AJ410074 AJ410075	AJ310797
Brachystelma franksiae N. E. Br.	South Africa: KwaZulu-Natal	*Nicholas 2846* (UDW)	AJ410022 AJ410023 AJ410024	—
Brachystelma macropetalum (Schltr.) N. E. Br.	South Africa	*Peckover sub Specks 2986* (MSUN)	AJ410025 AJ410026 AJ410027	AJ310782
Brachystelma nanum (Schltr.) N.E. Br.	South Africa	*Peckover sub Specks 2988* (UBT)	AJ410028 AJ410029 AJ410030	AJ310783
Brachystelma pygmaeum N. E. Br. subsp. *flavidum* (Schltr.) R. A. Dyer	South Africa: KwaZulu-Natal	*Ward s.n.* (UDW)	AJ410031 AJ410032 AJ410033	AJ310784
Brachystelma rubellum (E. Mey.) Peckover	Tanzania: Arusha Distr., Lake Chala	*I. & C. Hemp 2665* (UBT)	AJ410076 AJ410077 AJ410078	AJ310798
Brachystelma schizoglossoides (Schltr.) N. E. Br.	South Africa: Eastern Cape	*Nicholas 2815* (UDW)	AJ410034 AJ410035 AJ410036	—
Ceropegia abyssinica Decne.	Tanzania: Singida	*Specks 1052* (UBT)	AM491523* AM491524* AM491525*	AM493298*

Table 1. Continued.

Species	Origin	Voucher	EMBL acc. no. *trnT-L* spacer *trnL* intron *trnL-F* spacer	EMBL acc. no. ITS
Ceropegia albisepta Jum. & H. Perrier	Madagascar: Toliara	*Teissier 24* (UBT)	AM491526* AM491527* AM491528*	AM493299*
Ceropegia ambovombensis Rauh & Gerold	Madgascar: Ambovombé	*Rauh 74872* (HEID)	AM491531* AM491530* AM491529*	AM493300*
Ceropegia arabica H. Huber	Yemen	*IPPS 2852* (UBT)	AM491532* AM491533* AM491534*	AM493301*
Ceropegia aristolochioides Decne. subsp. *deflersiana* Bruyns	Yemen: N Taizz, Al-Qaidah	*Říčánek & Hanáček 246* (UBT)	AM491535* AM491536* AM491537*	AM493302*
Ceropegia bulbosa Roxb.	India: s. loc.	*Rowland (Butler C 726)* (UBT)	AJ488342 AJ488343 AJ488344	AJ488783
Ceropegia crassifolia Schltr.	South Africa: KwaZulu-Natal	ex hort. sub *Specks 191* (UBT)	AM491538* AM491539* AM491540*	AM493303*
Ceropegia cufodontii Chiov.	Ethiopia: Shewa Region, W Ambo	*Liede & Mere 3522* (UBT)	AM491541* AM491542* AM491543*	AM493304*
Ceropegia cumingiana Decne.	Philippines: Luzon, Mt. Makiling	*Liede 3250* (ULM, UBT)	AM491544* AM491545* AM491546*	AM493294*
Ceropegia denticulata K. Schum. ex Engl.	Kenya: Mt. Kasigau	*Masinde & Mere 870* (MSUN)	AM488349* AM488348* AM488347*	AM493291*
Ceropegia dichotoma Haw.	Spain: Canary Islands, Tenerife	*Mere s.n.* (MSUN)	AM488350* AM488351* AM488352*	AM493290*
Ceropegia distincta N. E. Br.	Tanzania: Amani	*Liede & Mere 3376* (UBT)	AJ488345* AJ488346* AJ488347*	AJ488784
Ceropegia filiformis (Burch.) Schltr.	South Africa: Northern Cape	*Bruyns 6683* (MSUN)	AM488355* AM488354* AM488353*	AM493289*
Ceropegia foliosa Bruyns	Yemen: N Yemen, W Gum'a	*Mangelsdorff Y23* (UBT)	AM488356* AM488357* AM488358*	AM493288*
Ceropegia gilgiana Werderm.	Tanzania: Singida, SW Itigi	*E. & M. Specks 1039* (UBT)	AM488361* AM488360* AM488359*	AM493287*
Ceropegia humbertii H. Huber	Madagascar: Antsiranana	*Mangelsdorff M22* (MSUN)	AM488362* AM488363* AM488364*	AM493286*
Ceropegia intermedia Wight	India: Tamil Nadu, Ebanad	*Bruyns 5903* (UBT)	AM488367* AM488366* AM488365*	AM493285*
Ceropegia juncea Roxb.	India: Tamil Nadu, S Nagercoil	*Říčánek & Hanáček 92* (UBT)	AJ128798 AJ128799 AJ128800	AJ488785
Ceropegia konasita Masinde	Kenya: Taita, Bura	*Masinde & Mere 851* (EA)	AM488368* AM488369* AM488370*	AM493284*

Table 1. Continued.

Species	Origin	Voucher	EMBL acc. no. *trnT-L* spacer *trnL* intron *trnL-F* spacer	EMBL acc. no. ITS
Ceropegia linearis E. Mey. subsp. *woodii* (Schltr.) H. Huber	ex hort.	*Meve s.n.* (UBT)	AM488374* AM488375* AM488376*	AM493292*
Ceropegia longifolia Wall.	Bhutan: Wangduephodrang	*Liede 3280* (UBT)	AM488371* AM488372* AM488373*	AM493283*
Ceropegia maccannii Ansari	India: Maharashtra	*Řičánek s.n.* (UBT)	AM488379* AM488378* AM488377*	—
Ceropegia meleagris H. Huber	Nepal: s. loc.	*ex Shirley s.n.* (MSUN)	AM488380* AM488381* AM488382*	AM493282*
Ceropegia meyeri-johannis Engl.	Kenya: Mt. Kasigau	*Meve 936* (K, MSUN)	AM488385* AM488384* AM488383*	AM493281*
Ceropegia monticola W. W. Sm.	China: Yunnan, Lunan Distr.	*Schneidt & Frazer 96-145* (UBT)	AM491586* AM490376* AM490377*	AM493306*
Ceropegia nilotica Kotschy	Kenya: Voi Distr., Mbololo	*Masinde 836* (EA)	AJ402117* AJ402138* AJ402141*	AJ402161
Ceropegia pubescens Wall.	Nepal: Anapurna, Komrong Hill	*Gerl 637* (UBT)	AM490380* AM490379* AM490378*	AM493280*
Ceropegia racemosa N. E. Br.	Tanzania: Arusha Distr., Lake Chala	*Liede & Meve 3355* (UBT)	AM490381* AM490382* AM490383*	AM493279*
Ceropegia robynsiana Werderm.	Kenya: s. loc.	*Pierce 1244* (EA)	AM490386* AM490385* AM490384*	AM493278*
Ceropegia rupicola Deflers	Yemen: S Manákhah	*Mangelsdorff Y13* (UBT)	AM490387* AM490388* AM490389*	AM493277*
Ceropegia sankuruensis Schltr.	Cameroon: Mt. Cameroon	*Meve 908* (MSUN)	AM490392* AM490391* AM490390*	AM493276*
Ceropegia saxatilis Jum. & H. Perrier	Madagascar: Antsiranana	*Mangelsdorff M21* (MSUN)	AJ410040* AJ410041* AJ410042*	AJ310786
Ceropegia simoneae Rauh	Madagascar: Toliara, Tsihombé	*Rauh 73113* (HEID)	AM490393* AM490394* AM490395*	AM493275*
Ceropegia striata Meve & Masinde	Madagascar: Antsirabe	*Rauh 75007* (HEID)	AJ410043 AJ410044 AJ410045	AJ310788
Ceropegia variegata Decne.	Kenya: N Maralal	*Masinde & Meve 871* (MSUN)	AM490398* AM490397* AM490396*	AM493274*
Ceropegia yemenensis Meve & Mangelsdorff	Yemen: Ussab al Ali Massiv	*Mangelsdorff Y24* (B, FR, UBT)	AM490399* AM490400* AM490401*	AM493293*

Results

What characterizes *Ceropegia*?

Flower morphology. Reinvestigating the morphology of all taxa analyzed molecularly and studying the generic circumscriptions presented for *Ceropegia* (Huber, 1957; Dyer, 1983; Bruyns, 1985; Meve, 2002), it becomes apparent that *Ceropegia* species possess the following significant characters/character states (Figs. 1A, 2): (1) inflorescences with at least a short peduncle (reduced only in a few highly derived taxa) and short, erect pedicels consisting of soft, watery, and non-lignified tissue; (2) corolla tubes longer than the lobes, basally inflated, apically tapering, inside with more or less stiff hairs (rarely absent, then tube inside slippery smooth); (3) corolla lobes mostly apically fused, leaving five small openings in the sinuses; (4) gynostegia and corona as in subtribe Stapeliinae (e.g., *Brachystelma*, *Caralluma* R. Br. s. str.; cf. Meve, 1998) but with staminal corona lobes fairly long and overtopping the anthers (Figs. 2B, E; except for *Ceropegia crassifolia* Schltr. p.p. and *C. dorjei* C. E. C. Fisch.); and (5) micromyiophilous pollination. The highly specialized and complex pitfalls trap small flies predominantly of the families Ceratopogonidae, Chloropidae, Milichidae, and Phoridae for pollination (Vogel, 1961; Masinde, 2004; Ollerton, Masinde, Meve & Whittington, unpublished data). Pitfall flowers of the *Ceropegia* type also occur in *Riocreuxia*. Apart from anatomical differences (pedicels with sclerenchymatic tissue and a parallel-veined corolla, both absent in *Ceropegia*) and gynostegial dissimilarities like a style-head of the marsdeniad type (Meve, 1998; Meve & Liede, 2004: fig. 3B), the independence of *Riocreuxia* is also corroborated by molecular data (Meve & Liede, 2004). *Riocreuxia* is now placed in the Ceropegieae subtribe Anisotominae, which is sister to Stapeliinae (Meve & Liede, 2004).

Vegetative morphology. *Ceropegia* is more diverse vegetatively than florally. The growth forms encompass small geophytic, erect, or twining herbs; leafy twiners or stem-succulents (partly without leaves); or true lianas of more than 10 m height. Annuals are absent. The root system can be fibrous (Fig. 1B), can consist of fleshy, thickened secondary roots that form fusiform or cylindric storage organs (Fig. 1C), or can be a tuber formed by the primary root, often together with the hypocotyl (Fig. 1D). The fleshy secondary roots appear either in dense clusters (Fig. 1C) or are scattered over the root system. Normally, fleshy secondary roots and root tubers exclude each other (Fig. 1B–D). The three basal character states of the roots and stem succulence or non-succulence are included in Figure 3 because they are important in recognizing groups of closely related taxa. The formation of hypocotyl/root tubers is reported for 80 of the ca. 180 species. Fusiform-fleshy secondary roots are known from ca. 50 species (cf. Meve, 2002). The lack of any underground storage organ is characteristic for another ca. 50 species. The most derived, tuberous growth form is restricted to clades C and G of the seven main clades (Fig. 3). Significantly, in clade A fibrous non-tuberous roots occur in all four species of this most early-branching clade, whereas in clade F, the Asian–Canary clade, the species of the truly Asian subclade, the *C. meleagris* H. Huber–*C. monticola* W.W. Sm.–*C. longifolia* Wall. clade (clade D), are equipped with fusiform-fleshy roots only. In contrast, the fusiform-fleshy type of root is completely absent in Madagascar (clade G2, Fig. 3).

Cytology. Numerous chromosome numbers for *Ceropegia* were included in the overview of the family by Albers and Meve (2001). Additional counts for East African taxa were contributed by Masinde (2000). All published numbers can be viewed at: <http://www.pflanzensystematik.uni-bayreuth.de.> Meanwhile, additional counts have increased the number of chromosomally known species of *Ceropegia* to 74 or ca. 40% of the genus (Meve, unpublished data). For all taxa investigated, the basal chromosome number is $x = 11$. Diploids with $2n = 22$ dominate by far. Only a few taxa are characterized by a tetraploid genome of $2n = 44$ chromosomes. These are the leafy East African *C. linearis* subsp. *woodii*, *C. inornata* P. R. O. Bally ex Masinde, and *C. cufodontii* Chiov. (Meve, unpublished data); the Madagascan representatives *C. ampliata* E. Mey. and *C. racemosa* N. E. Br.; and the stem-succulent Indian species *C. juncea* Roxb. The chromosome numbers of the taxa investigated molecularly are plotted on the cladogram (Fig. 3). The majority of species have medium-sized chromosomes around the average length found for the whole tribe, which is 1.01 µm/chromosome (Albers & Meve, 2001). However, the average chromosome sizes in *Ceropegia* vary considerably between 0.81 µm and 1.58 µm (Meve, unpublished data). The largest chromosomes are found in leafy ceropegias with fibrous roots (e.g., *C. sankuruensis* Schltr. and *C. cumingiana* Decne., with around 1.40 µm average length) except for the stem-succulent Canary Islands species, *C. dichotoma* Haw., where the chromosomes measure only 0.87 µm on average.

DNA analysis

The cpDNA data set. The cpDNA alignment comprises 69 taxa and a total of 1973 characters

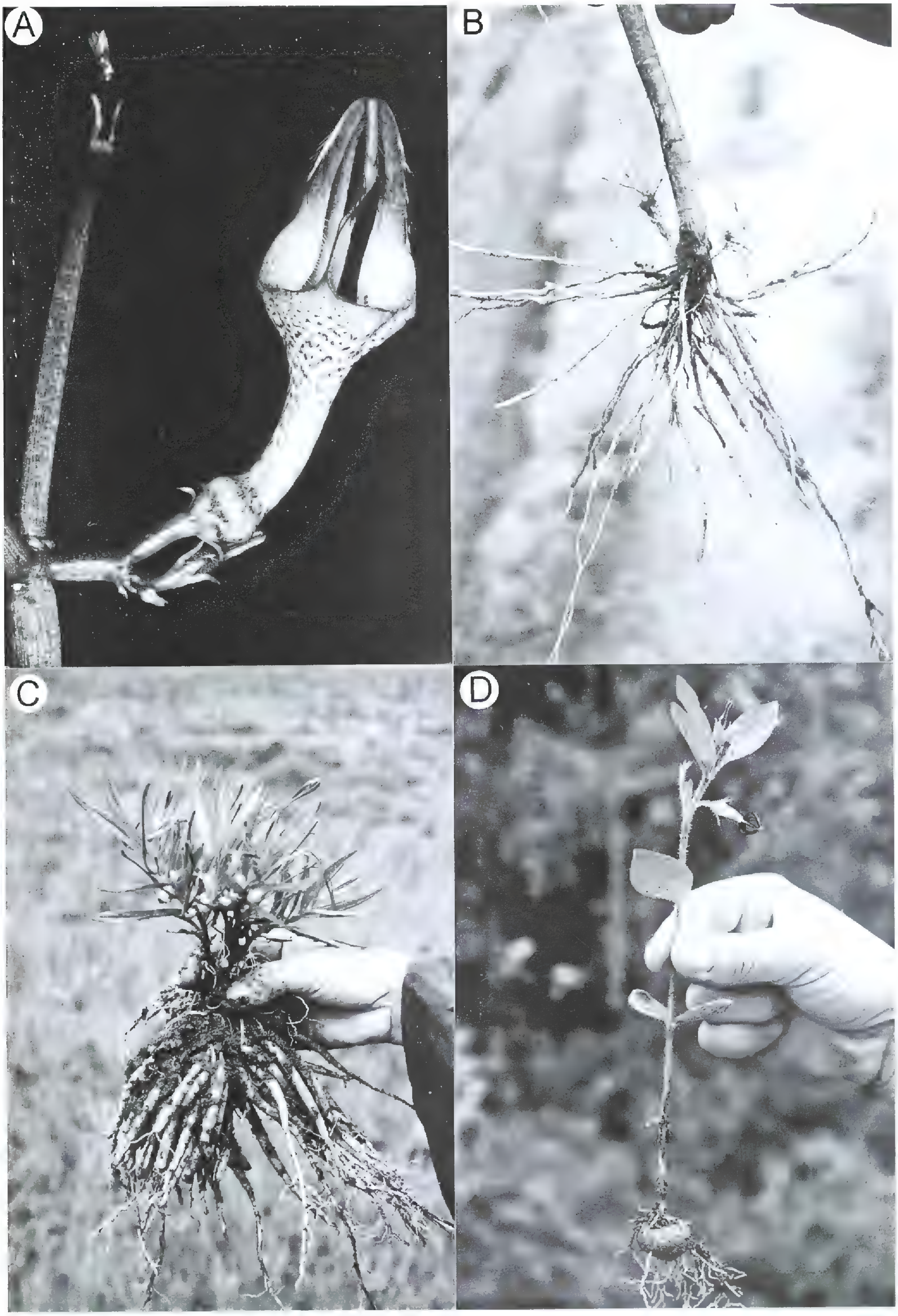

Figure 1. *Ceropegia*. —A. Flowering stem of *C. juncea*. —B. Stem base of *C. lugardiae* showing the fibrous roots. —C. Whole plant of *C. gilgiana* with cluster of swollen, fusiform roots. —D. Whole plant of *C. abyssinica* showing the root tuber. A: *Řičánek & Hanáček 92*; B: *Specks 1042*; C: *Specks 1039*; D: *Specks 1039*. Photo: A by U. Meve; B–D by E. Specks.

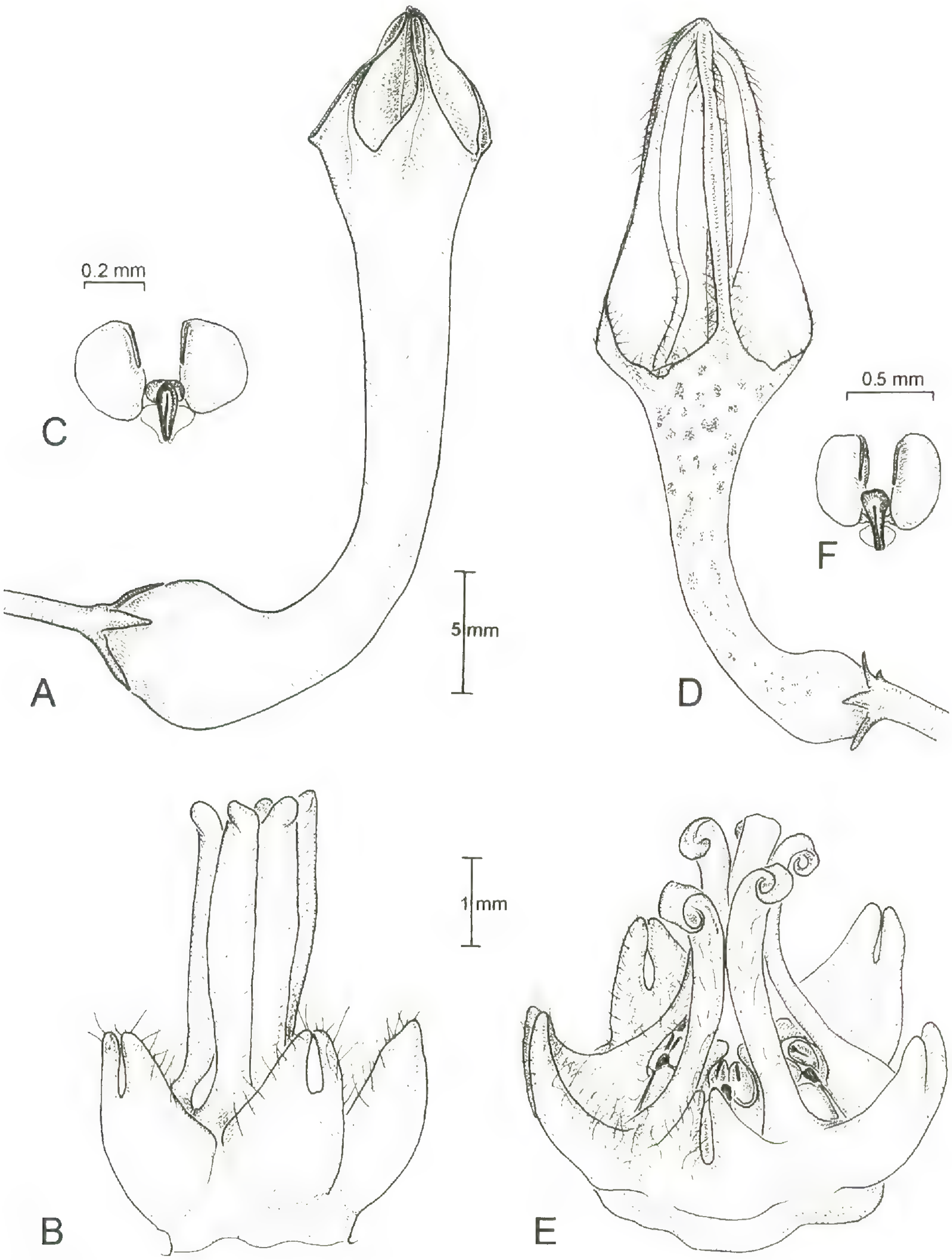

Figure 2. A–C. *Ceropegia aristolochioides* subsp. *defflersiana*. —A. Flower. —B. Corona. —C. Pollinarium. D–F. *Ceropegia juncea*. —D. Flower. —E. Corona. —F. Pollinarium. A–C: *Hangelsdorff 11* (UBT); D: *ex hort. P. Shirley* (UBT). E F: *Řičánek & Hanáček 92* (UBT). Drawn by U. Meve.

(1013 sequence characters in the *trnT-L* spacer [primers a and b], 551 sequence characters in the *trnL*-intron [primers c and d], and 409 sequence characters in the *trnL-F* spacer [primers e and f]); a total of 26 data cells are unknown. This alignment, as well as that for ITS, are available from the authors or can be viewed at <http://www.pflanzensystematik.uni-bayreuth.de> or in TreeBASE (study accession number = S1710, matrix accession numbers = M3095 and M3096; Sanderson et al., 1994). Parsimony analysis of the cpDNA sequence characters alone (112 parsimony informative characters) resulted in more than 79,500 most parsimonious trees of 184 steps with consistency index (CI) of 0.72 (excluding uninformative characters) and retention index (RI) of 0.92.

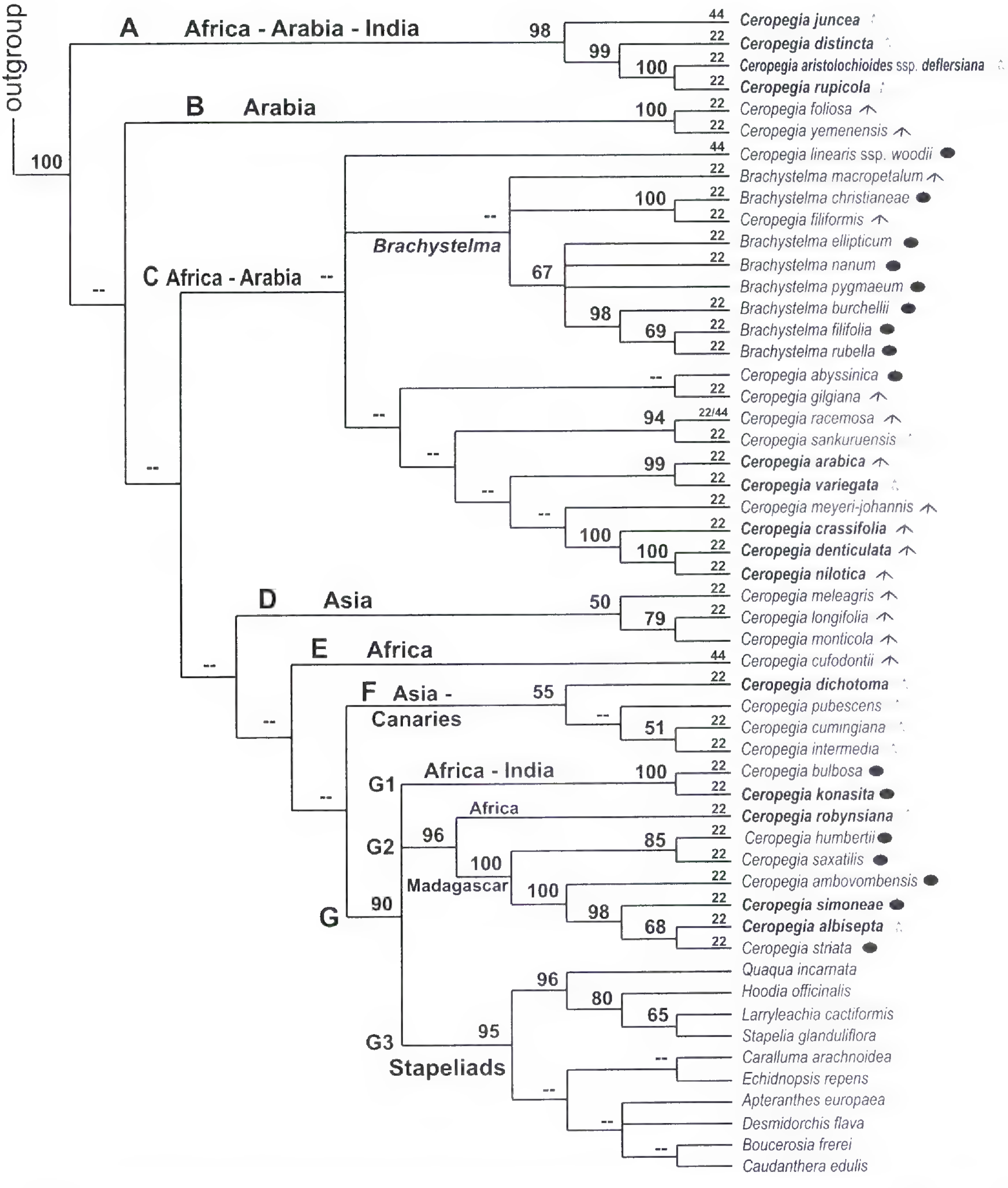

Figure 3. Strict consensus tree of 234 most parsimonious trees derived from cladistic analysis of combined ITS and *trnT-L* and *trnL-F* spacers, and the respective indels coded as binary characters (l = 1118 steps, CI = 0.5519 [excluding uninformative characters], RI = 0.8127, rescaled consistency index [RC] = 0.4458; missing data [for ITS] have been coded with "nn"). Numbers indicate bootstrap percentages: – indicates bootstrap percentage < 50%; numbers on the end branches indicate the 2*n* chromosome number of the taxon where available; stem-succulent *Ceropegia* taxa are printed in **bold**; ↑ = roots fibrous; ↟ = roots fleshy-fusiform; ● = roots tuberous.

In previous analyses (Meve & Liede, 2002a, 2004) and the one presented here, the Ceropegieae are monophyletic and form a strongly supported clade (100% bootstrap support), as does the terminal subtribe Stapeliinae. Within the latter, resolution in the cpDNA analysis alone is rather low (not shown, available from authors on request). Together with 12 mostly Asian *Ceropegia* species, the stapeliads remain unresolved. Most conspicuous is the subclade formed by the endemic Madagascan *Ceropegia* taxa plus *C. robynsiana* Werderm., and a clade in which all investigated *Brachystelma* species and 12 Afro-

Arabian *Ceropegia* species are combined. However, all these clades retrieved are without statistical support or nearly so.

The ITS data set. The alignment for ITS comprises 65 taxa and a total of 713 sequence characters. Three sequences could not be generated, one was not aligned (see Materials and Methods), and seven data cells are unknown. Parsimony analysis of ITS data alone, excluding the four missing sequences (299 parsimony informative characters), resulted in more than 65,400 trees of 914 steps (not shown). CI (excluding uninformative characters) is 0.53, and RI is 0.79.

Analysis of the ITS data set (not shown, available from authors on request) results in a better-resolved tree with regard to the subclades. The whole *Ceropegia* complex remains unresolved. The stapeliads switch to a subclade with 92% bootstrap support. *Brachystelma* forms a moderately supported subclade (75% BP) within a large basal polytomy, which includes *C. filiformis* (Burch.) Schltr. The endemic Madagascan taxa, again, form a monophyletic group, now supported by 100% BP. There are several cases where sister species relationships are now supported by 100% BP (as is the same in the combined analysis, Fig. 3): *B. christianeae* Peckover and *C. filiformis*; *C. foliosa* Bruyns and *C. yemenensis* Meve & Mangelsdorff; *C. crassifolia*, *C. denticulata* K. Schum. ex Engl., and *C. nilotica* Kotschy; *C. rupicola* Deflers and *C. aristolochioides* Decne.; and *C. bulbosa* Roxb. and *C. konasita* Masinde.

The combined cpDNA and nrDNA data set. The partition homogeneity test (Cunningham, 1997) suggested that combining the data sets would generally improve phylogenetic accuracy ($P = 0.02$). Parsimony analysis of ITS combined with the *trnT-L* and *trnL-F* spacer regions (409 parsimony informative characters) resulted in 234 trees of 1118 steps, with CI (excluding uninformative characters) of 0.55 and RI of 0.81 (Fig. 3). In this analysis, the species with missing ITS sequences have been eliminated.

Of all data sets analyzed, the combined data set (Fig. 3) shows the highest resolution and, in general, better support for clades retrieved in the individual cpDNA and ITS analysis. The statistical support of the subclade showing the monophyly and the sister relationship of the Madagascan taxa to the East African *Ceropegia robynsiana* is strong (96% BP). The purely Madagascan subclade (100% BP) falls into two subclades. The first is a moderately supported subclade (85% BP) containing the leafy and tuberous *C. humbertii* H. Huber and *C. saxatilis* Jum. & H. Perrier; the other is a strongly supported subclade (100% BP) comprising a diverse blend of taxa representing all main growth forms described for Madagascar endemics. The strongly supported clade A (98% BP) comprising the non-tuberous *C. juncea*, *C. aristolochioides* subsp. *deflersiana* Bruyns, *C. distincta* N. E. Br., and *C. rupicola* now appears at the base of the cladogram as sister to the rest of Stapeliinae. There is no statistical support for most of the remaining main clades. The large clade C, shared by 12 *Ceropegia* and all *Brachystelma* species investigated, is nested in the middle of the ingroup, making *Ceropegia* without *Brachystelma* paraphyletic. In the terminal clade G (90% BP), the well-supported subclades G1 (*C. bulbosa*–*C. konasita*, 100% BP), G2 (*C. robynsiana* plus the Madagascan group, 96% BP), and G3 (stapeliads, 95% BP) form a terminal polytomy. Thus, *Ceropegia* without the stapeliads is paraphyletic in this second case. However, all three subclades of clade G represent strongly supported monophyletic groups (Fig. 3). *Ceropegia* thus forms a grade of seven clades (A–G) pointing to complex infrageneric relationships. *Ceropegia* species mostly cluster according to geographical distribution; in some cases, the growth form serves as a good marker for close relationship (Fig. 3: clades A, D, F). Unexpectedly, the Canary Island species, *C. dichotoma*, shares a clade with three taxa from India and Australasia (clade F), although statistical support is low (55% BP). *Ceropegia juncea*, restricted to India where it is morphologically isolated by its succulent stems and reduced leaves, shares clade A with East African–Arabian taxa (85% BP; Fig. 3).

Discussion

Chromosome numbers show little variation at the genus level in *Ceropegia*. However, at species level they are significant. The occurrence of diploid specimens of *C. ampliata* and *C. racemosa* in Africa and tetraploid specimens in Madagascar has been interpreted as proof of long-distance dispersal from Africa to Madagascar, because polyploidy is a unidirectional event (Meve & Liede, 2002b). The other few known polyploids seem to occur randomly scattered over South Africa, East Africa, and India (cf. Fig. 3). Also noteworthy is the trend toward larger chromosomes in the non-tuberous species; this has been demonstrated in the studies of Albers and Meve (2001) in Periplocoideae, Secamonoideae, and Asclepiadoideae, which show a general trend from larger chromosomes in basal taxa to smaller chromosomes in the most advanced tribe, Asclepiadeae. *Ceropegia dichotoma*, endemic to the Canary Islands, has unusually small chromosomes within the genus and could then be interpreted as possibly the most

advanced species sampled in this study. Bruyns (1986) regarded some floral characters of this species, such as the poorly or uninflated corolla tube or the small gynostegium, as primitive. This interpretation, however, is not supported in our phylogeny (clade F, Fig. 3). Interestingly, *C. dichotoma* is in a weakly supported clade (55% BP) with the Asian, non-succulent, fibrous-rooted taxa *C. pubescens* Wall., *C. intermedia* Wight, and *C. cumingiana*. *Ceropegia dichotoma*, as well as its sister species *C. fusca* Bolle, therefore might have reached the Canary Islands more recently, where the stem-succulent growth then evolved in response to the special semi-arid and volcanic environment. This growth form, the lack of leaf petioles and peduncles, the small chromosomes, and the floral pecularities mark it as a highly specialized species that evolved in island isolation. This combination of characters is not known otherwise in the genus. Elsewhere, *Ceropegia* has shown considerable radiative potential (e.g., in Madagascar), whereas in the Canary Islands it is restricted to *C. dichotoma* (including two subspecies) and *C. fusca*, all of which point toward a rather recent arrival of *Ceropegia* on the Canaries.

Molecular phylogeny and vegetative morphology are often linked to geography (cf. Fig. 3). A morphological exception is the unexpected sister relationship of *Ceropegia filiformis* and *Brachystelma christianeae*, since *C. filiformis* has fusiform-fleshy roots and typical *Ceropegia* pitfall flowers, whereas *B. christianeae* has a single tuber and flat, radiate flowers. However, *B. christianeae* does not have the pubescent pedicels and abaxial corolla typical for *Brachystelma*; conversely *C. filiformis* has a swollen style-head that projects above the anthers, which is unknown in *Ceropegia* but occurs occasionally in *Brachystelma* (Bruyns, 1995). Thus, neither species fits within the general concepts of their respective genera, but, in contrast, they also do not share morphological features indicating close relationship apart from their very small habit and wiry stems. Nevertheless, their sister relationship is strongly supported here (100% BP, Fig. 3) and is also supported by shared indels, which cannot be easily ignored taxonomically. Morphological transitions between *Ceropegia* and *Brachystelma* are not restricted to this case, however. There are other borderline cases, such as *B. mafekingense* N. E. Br. and *B. gymnopodum* (Schltr.) Bruyns, which have been moved between *Ceropegia* and *Brachystelma* in the past (cf. also Bruyns, 1995). In all these cases, introgression via interspecific and intergeneric hybridization events are most likely responsible for these patterns. Because small flies (see Results: Flower Morphology, above) are involved in the pollination of both *Brachystelma* and *Ceropegia* and because many species of each genus share similar floral attractants (Meve & Liede, 1994b), the most important prerequisites for cross-pollination are already in place.

Taxonomically, inclusion of *Brachystelma* (ca. 120 species) in *Ceropegia* (180+ species) would solve part of the problem; however, the morphological concept of *Ceropegia* (see below) would be demolished, and the many name changes would be unpleasant. Nevertheless, this option should be kept in mind. Before such a step is undertaken, however, additional *Brachystelma* species, especially from Asia, should be included in sequence analysis.

Non-tuberous species occur in all the main clades retrieved (Fig. 3). As far as resolution allows, these taxa then typically appear in less-derived positions. This is true for the terminal polytomy of clade G (with *Ceropegia robynsiana*); the intermediate polytomy of clade C, which includes *Brachystelma* (with *C. sankuruensis*); the subclade F as sister to the terminal polytomy (with *C. dichotoma*); and the subclade A as sister to all Stapeliinae with all species (*C. juncea*, *C. distincta*, *C. aristolochioides*, and *C. rupicola*). Fibrous roots, always present in the stem-succulent stapeliads, are recognized as a symplesiomorphy for the Stapeliinae and also for the tribe Ceropegieae, because the basal three subtribes (Heterostemminae, Anisotominae, and Leptadeniinae; Meve & Liede, 2004) also lack root succulence, except for slightly swollen secondary roots in *Sisyranthus* (Anisotominae). This sounds trivial, but in the most basal tribe of Asclepiadoideae, the Fockeeae (Verhoeven et al., 2003), root tubers are the rule. The minority of *Ceropegia* species have such plesiomorphic fibrous roots (see above; Fig. 3). Concerning stems and leaves, non-succulence, again, must be regarded as plesiomorphic because all other and basal representatives (the subtribes Heterostemminae, Anisotominae, and Leptadeniinae) have "normal," sometimes slightly woody stems and mesophyllous, sometimes slightly xerophytic leaves. Indian species such as *C. intermedia* or *C. pubescens*, as well as the Australasian *C. cumingiana*, also share these morphologically less-specialized features, although they are found in an intermediate position in our phylogeny (clade F). Although flower morphological parallelisms (homoiologies) in response to pollinator pressure should always be taken into consideration in the asclepiads (e.g., Meve & Liede, 1999, 2002a), the similarities in the morphology of the gynostegia, coronas, and pollinaria of *C. juncea* and, for example, *C. aristolochioides* subsp. *deflersiana* (Fig. 2) are striking. In particular, the deltoid corpuscle projections and pollinia possessing rather triangular and more or less membraneous margins (germination crests) are mor-

phologically significant for this group (clade A, Fig. 3). Taxa such as *C. somalensis* Chiov., *C. yorubana* Schltr., and *C. zambesiaca* Masinde & Meve should be included in this group based on morphological grounds. These morphological characters are useful systematic markers here, especially since they are congruent with the results of molecular analysis. The situation is similar to that found in *C. dichotoma* and, for example, *C. pubescens* (clade F, Fig. 3). Although these two taxa are vegetatively quite different, the peculiar structure of their gynostegium and corona, in which the interstaminal coronas are almost completely reduced and fused to the backs of the staminal corona (cf. figs. in Bruyns, 1986), is very similar. These corona pecularities also appear in other Asian taxa such as *C. hookeri* C. B. Clarke ex Hook. f. However, other members in this non-tuberous group of taxa (clade A) do not share this type of corona, so that interpretation as a homoplasy cannot be excluded.

SUCCESS IN GAINING A MOLECULAR PHYLOGENY AND ITS TRANSLATION INTO TAXONOMY

Inclusion of more species into the molecular phylogeny and, perhaps, the use of an additional molecular marker might improve the resolution and the statistical support of the *Ceropegia* cladogram(s). However, there is reasonable doubt that the result would lead to a phylogeny more easily transposed into taxonomy than the one presented here. Quite possibly, the number of clades would increase further. As could be expected when starting an analysis of a large and widely distributed genus such as *Ceropegia*, the cladograms constructed from the molecular data do not answer all the questions. Worse yet, numerous questions arise where there were none before. *Ceropegia* species appear in seven different clades; the six most early branching clades form a grade and include *Brachystelma* (Fig. 3, clade C), while the remaining clades are part of a terminal polytomy that falls into three well-supported subclades and also includes the stapeliad clade. The most early branching clade A, and only this clade, includes representatives from all main distribution areas (Africa, Arabia, Asia) except for Madagascar. Thus, an area of origin for *Ceropegia* can hardly be determined. However, East African/Arabian taxa dominate in clade A, which is followed, again, by two clades containing only African/Arabian taxa, so that the available data could be interpreted as support for a northeast African center of origin. This area has also been determined to be the primary evolutionary center for the stapeliads, in particular, *Caralluma* s. str. (Meve, 1997; Meve & Liede, 2002a). Restricted to just two clades, D and F, several Asian taxa appear, in contrast to the isolated *Ceropegia juncea* in clade A; however, there is no substantial statistical support for these clades. This grouping might indicate that the recent Asian taxa have developed more or less independently from the rest of the genus for a longer time, and that the origin of the genus cannot be located in Asia. These nearly exclusively Asian clades, nevertheless, include one taxon from the western distribution area, the Canary Islands endemic *C. dichotoma*. This indicates that the Canary Islands taxa, surprisingly, have no close African relative left when long-distance dispersal from West African progenitors is supposed, although accessory species in the analysis might change this impression. The endemic Madagascan taxa are grouped together on subclade G2 (Fig. 3) and are sister to *C. robynsiana*. Madagascar, therefore, has been obviously invaded just once (excluding *C. ampliata* and *C. racemosa*, see below) and comparatively late by a *Ceropegia* related to East African species such as *C. robynsiana*, which then served as progenitor of today's phenetically rather diverse but genetically closely interrelated Madagascan stock. A classification restricting section *Dimorpha* to stem-succulent Madagascan taxa alone (Meve & Liede, 1994a), therefore, should be rejected. Instead, section *Dimorpha* should cover all *Ceropegia* species endemic to Madagascar, including *C. robynsiana*. However, this section then would seem to have no morphological synapomorphy left. The situation in *Ceropegia* on Madagascar, with a late arrival by rather derived taxa followed by considerable diversification, is reminiscent of the situation in the highly diverse and variable genus *Impatiens* L. (Balsaminaceae), where the most basal taxa are found in South and Southeast Asia (Yuan et al., 2004). The two nonendemic *Ceropegia* species also found in Madagascar, *C. ampliata* and *C. racemosa*, have been identified as not being closely related to the Madagascan stock (Huber, 1957; Meve & Liede, 1994a). Both are tetraploid in Madagascar, diploid in Africa (Meve & Liede, 2002b), and nested within their African relatives of clade C (molecularly tested for *C. racemosa* only). Thus, all three of these data sets strongly suggest that both *Ceropegia* species reached Madagascar rather recently by means of long-distance dispersal (Meve & Liede, 2002b).

Finally, the well-supported position of the sisters, *Ceropegia bulbosa* from India and *C. konasita* from Africa, on the terminal polytomy (clade G) remains unexplained, but suggests another colonization of Asia by African stock. Infrageneric patterns are too reticulate morphologically to allow revision at the sectional level. Single groups of taxa (e.g., clades A, F, and subclade G2; Fig. 3) represent good candidates for sections should sectional classification be appropriate later when more data become available.

Brachystelma (ca. 120 species) and the stapeliads (ca. 400 species) are nested within different lineages of *Ceropegia*, therefore rendering it paraphyletic. Transferring the cladification presented here into classification would mean either reducing the subtribe Stapeliinae to one single genus or splitting *Ceropegia* into seven or eight genera (with *Brachystelma* included in a genus representing clade C). Neither the first nor the second solution is a desirable option. Either way, none of these newly created genera could be characterized by a specific set of morphological character states, and almost none could be keyed out morphologically. Considering the rather convincing current taxonomy, as well as the many phenetic differences between and, most important, within the groups involved, such translation of cladistics into classification is rejected here. As Brummitt (1996) and Grant (2003) pointed out, the paraphyly concept belongs to cladistics and not to taxonomy. This statement is especially pertinent when the cladistic results are based on the analysis of few non-genomic markers and where they are highly incongruent with the morphological characters commonly used in taxonomy. Diverse molecular studies in Asclepiadoideae have provided many new insights into possible phylogenies and relationships, and paraphyletic groups have been identified in several cladistic analyses as well. Sometimes these paraphyla contradicted the then-current taxonomy at first glance, but reconsideration of morphological data, for example, revealed the traditional view to be one-sided. For example, the former genera *Sarcostemma* R. Br., *Karimbolea* Desc., *Folotsia* Costantin & Bois, and *Platykeleba* N. E. Br. were taxonomically separated from similarly leafless and stem-succulent *Cynanchum* L. for decades because of their aberrant corona structure. Molecular analysis (Liede & Kunze, 2002; Liede & Täuber, 2002), however, has shown that, without exception, all of these stem-succulent taxa form a well-supported, monophyletic subgroup within *Cynanchum*, a plausible result that is corroborated by morphological (stems and leaves), anatomical (Liede & Kunze, 2002), and also biogeographical data, since Meve and Liede (2002b) have shown that this group originates in Madagascar. The case presented in the present paper, however, does not offer such a "smooth" solution, possibly because this study deals with a very fast-evolving genus (cf. Klak et al., 2004), in which hybridization events result in a reticulate molecular phylogeny (cf. Sosef, 1997). As long as molecular and "classical" data remain incongruent to the degree exhibited in *Ceropegia*, paraphyly must be acceptable for taxonomists aiming to produce a classification that allows recognition of the organisms. Recently, in an open letter to the editor of the journal *Taxon* signed by over a hundred taxonomists, the appeal has emphatically been made that "Paraphyly should be accepted" (Nordal & Stedje, 2005). *Ceropegia*, as has been shown here, represents a group of closely related and interrelated taxa, but its recognition as a monophyletic taxon failed. The taxa are nevertheless held together by a wide array of specific morphological characteristics. In this special situation, we feel it is most prudent to uphold the current taxonomy.

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DIVERSIFICATION OF ASCLEPIADOIDEAE (APOCYNACEAE) IN THE NEW WORLD[1]

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Abstract

Plastid sequences of *trnL-F* are used to estimate the age of biogeographical events in New World Asclepiadoideae. These data suggest that the subfamily arose somewhere in the Old World in the Late Eocene (40 million years ago (Ma)) and that there were at least four independent episodes of dispersal to the New World. The MOG clade, comprising Metastelmatinae, Oxypetalinae, and Gonolobinae, probably colonized South America through transoceanic dispersal from Africa at 32 Ma. The three subtribes emerged at 24 Ma, radiating 15–16 Ma. *Cynanchum* L. subg. *Mellichampia* (A. Gray) Woodson (Cynanchinae) only arrived in the New World at 24 Ma and has since diversified gradually. *Asclepias* L. (Asclepiadinae) probably arrived in the New World through the Bering Strait at 20 Ma, dispersing to South America before the emergence of the Isthmus of Panama. More recently, tropical *Marsdenia* R. Br. (Marsdenineae) probably arrived in the New World by long-distance dispersal at 16 Ma. Most diversification in New World Asclepiadoideae is estimated to have occurred during the Miocene. The synchrony of events suggests an environmental influence on the diversification of the lineages. Nevertheless, individual rates of diversification point out the importance of intrinsic factors.

Key words: Apocynaceae, Asclepiadoideae, biogeography, molecular dating, Neotropics, phylogeny, systematics.

The Asclepiadoideae comprise ca. 3000 species, around 40% of them in the New World (Meve, 2002). Although the Neotropics is one of the centers of diversity for Asclepiadoideae, little has been done to understand the evolution of this subfamily in the region. Most studies were restricted to traditional taxonomy, and classifications have been pragmatic and artificial (Rapini, 2002). During the turn of the millennium, however, the introduction of cladistic studies, especially those based on molecular phylogenetics, reflected a changing perspective toward the classification of Asclepiadoideae (Rapini et al., 2003, and references therein). Despite this progress, only a few studies (e.g., Goyder, 2006) have explored the biogeography of the subfamily.

The hypothesis of African origin for the Asclepiadoideae has long been assumed, predicted by their high diversity, both in the number of species and higher taxonomic levels in Africa, and confirmed by the phylogeny of the group, which is marked by a basal grade composed of Old World, predominantly African, taxa (Sennblad & Bremer, 1996; Civeyrel et al., 1998; Potgieter & Albert, 2001; Liede, 2001; Rapini et al., 2003). The New World Asclepiadoideae are divided into four lineages (Rapini et al., 2003). The largest one has been denominated MOG and is composed of Metastelmatinae, Oxypetalinae, and Gonolobinae, plus the recently recognized subtribe Orthosiinae and a basal grade formed by *Pentacyphus* Schltr. and *Diplolepis* R. Br., respectively (Liede-Schumann et al., 2005). Two other New World groups, namely *Cynanchum* L. subg. *Mellichampia* (A. Gray) Woodson (Cynanchinae) and *Asclepias* L. (Asclepiadinae), are nested within the predominantly Old World MOG's sister clade referred to as ACT, which comprises the subtribes Asclepiadinae, Cynanchinae, and Tylophorinae (Rapini et al., 2003). The fourth clade is the New World *Marsdenia* R. Br. (Marsdenieae), the only group bearing erect pollinia; the other three clades belong to the Asclepiadeae and possess pendent pollinia.

Despite references to Apocynaceae (e.g., LaMotte, 1952; Brown, 1962) that include the extant genus *Alyxia* Banks ex R. Br. (Müller, 1981) in the Paleocene, molecular data (Wikström et al., 2001) and the most reliable fossil evidence of their earliest appearance (Magallón et al., 1999) have established the origin of Apocynaceae in the Early Eocene (ca. 53 million years ago (Ma)) or later (ca. 45 Ma; Magallón & Sanderson, 2001; Wikström et al., 2001). Nested within Apocynaceae, Asclepiadoideae have a later origin, having few known land routes available to explain their dispersals from the Old

[1] AR thanks Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support for attending the Congress. AR and CvdB are supported by a grant (PQ2 and PQ1D, respectively) from CNPq.

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World into the New World. The aim of this study is to explore current distribution and phylogenetic relationships of the New World Asclepiadoideae in order to make inferences about their origin and diversification.

Material and Methods

We attempted to sample all species of New World Asclepiadoideae employed in molecular phylogenetic studies. The data matrix consisted of 216 sequences of *trnL* intron and *trnL-F* intergenic spacer (*trnL-F*), the most used markers at this taxonomic level in Asclepiadoideae. Most asclepiad sequences (175) were previously used in Rapini et al. (2003) and Liede-Schumann et al. (2005), but a few sequences were added (e.g., Liede & Täuber, 2002; Meve & Liede, 2004; Rapini et al., 2006) to improve sampling in particular regions of the tree. Gelseminaceae and Loganiaceae were collectively used as outgroups; samples from other subfamilies of Apocynaceae, namely Apocynoideae, Rauvolfioideae, Periplocoideae, and Secamonoideae were also included (Appendix 1).

Bayesian analysis was carried out in MrBayes 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The substitution model selected was GTR+G, estimated through hierarchical likelihood ratio tests (hLRT) in MrModeltest 2.2 (Nylander, 2004) as the one that better fits our data. We ran 1,000,000 generations, sampling a tree every 100 generations. The majority rule consensus was then built in PAUP* 4.0b10 (Swofford, 2001) using trees of the two runs (burn-in trees excluded) to generate the object tree. The topology obtained was then used for maximum likelihood branch-length optimization using PAUP* and the general model, and parameters previously estimated in MrModeltest. Molecular clock assumption was tested and rejected by likelihood ratio tests (LRT). Based on the branch-lengths, we then estimated absolute and relative rates for the tree through nonparametric rate smoothing (NPRS) using the software r8s (Sanderson, 1997). Based on a compromise between fossil and molecular lines of evidence (above), we converted relative to absolute ages calibrating the Apocynaceae crown group in the Paleocene/Eocene boundary (54 Ma). However, an alternative calibration in the Apocynaceae stem group at the same period was also considered.

Results

The data matrix for the plastid *trnL-F* (available upon request from AR) included 1327 characters (146 characters of dubious alignment were excluded). The cold chain in the two independent runs reached stabilization of likelihood values at about 80,000 generations. We discarded 10% of the trees (burn-in) in each run, summarizing the data based on the remaining 9001 trees of the two runs.

Previous phylogenetic studies (Rapini et al., 2003, 2006; Liede-Schumann et al., 2005) have already discussed topological relationships among these groups. Here, we restrict the results to the information present in the chronogram (Figs. 1–5). Differences between the two calibrations (crown and stem group) do not exceed 10 million years in most basal nodes of Asclepiadoideae, and the precision of estimates decreases with the distance from the calibration point. Therefore, these differences do not greatly affect overall biogeographical interpretations of New World Asclepiadoideae events, and the alternative calibration in the Apocynaceae stem group is omitted below.

With our calibration of the Apocynaceae crown group at 54 Ma, the Asclepiadoideae emerged at 40 Ma, the Asclepiadeae at 35 Ma, and the Marsdenieae and Ceropegieae at 30 Ma. The New World clade of *Marsdenia* would have appeared after 16 and radiated at 9.9 Ma (Fig. 1).

In Asclepiadeae, the Astephaninae arose at 33 Ma but became diverse only at 4.9 Ma. MOG and ACT clades (see introduction for definitions) diverged at 32 Ma. ACT diversified, giving rise to important groups (Tylophorinae, Asclepiadinae, and Cynanchinae) at around 27 Ma. The New World *Asclepias* diverged from African Asclepiadinae at 20 Ma. The South American group (including *A. curassavica* L.) arose between 16 and 7.5 Ma (Fig. 2). The New World *Cynanchum* (subg. *Mellichampia*) appeared at 24 Ma, diverging between North and South American species around 20 Ma (Fig. 3).

In the MOG clade, basal genera *Diplolepis* and *Pentacyphus*, as well as Orthosiinae and the MOG core group, appeared at 27 Ma. The Orthosiinae had events of diversification at 17 and 14 Ma, with a secondary diversification in *Orthosia* Decne. at 3.7 Ma. The MOG core group diversified at 24 Ma, having *Funastrum* E. Fourn. radiating at 16 Ma, *Tassadia* Decne. at 7 Ma, and the Gonolobinae at 15 Ma (Fig. 4). The Oxypetalinae became diverse at 15 Ma, with secondary events of diversification in *Philibertia* Kunth and *Oxypetalum* R. Br. at about 8 Ma (Fig. 5A). The Metastelmatinae became diverse only at 12 Ma, with secondary events of diversification in *Minaria* T. U. P. Konno & Rapini at 4.7 Ma and in the *Blepharodon* Decne.–*Hemipogon* Decne. clade at 2.6 Ma (Fig. 5B).

Absolute rates of diversification for clades in New World Asclepiadoideae ranged from 0.0407 speciation events per million years in *Pentacyphus* to 0.3245 in the sister group of *Blepharodon* s. str. Among the

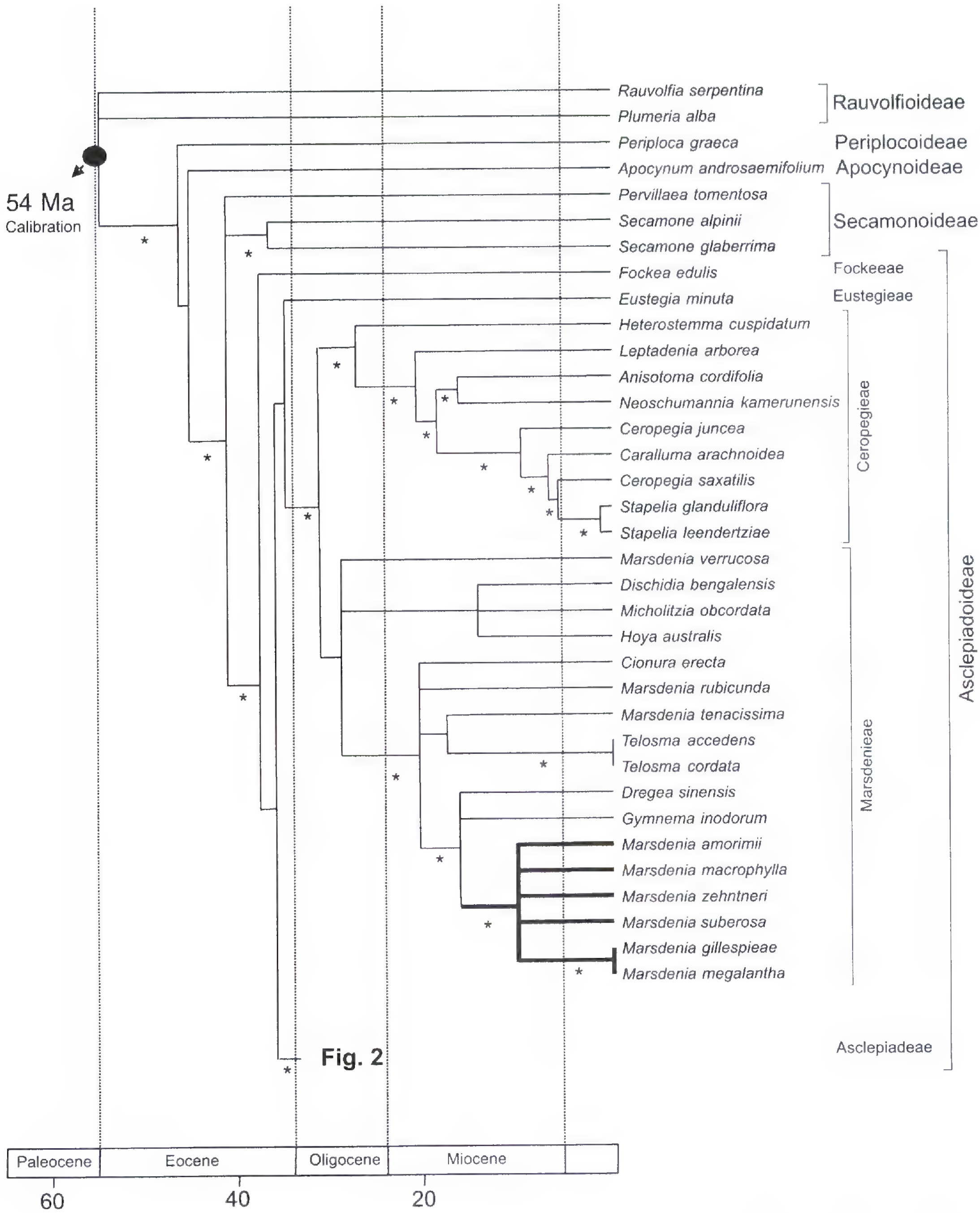

Fig. 2

Figure 1. Lower portion of the Apocynaceae chronogram. New World clades are in bold lines. Asterisks (*) identify clades with posterior probability greater than 94%. Scale units are million years ago (Ma).

New World lineages of Asclepiadoideae, *Marsdenia* presented the highest rate of diversification (0.2655) and *Cynanchum* subg. *Mellichampia* the lowest (0.1306). *Asclepias* and the MOG group presented intermediary rates (0.2414 and 0.2075, respectively), the former similar to that in MOG core group (0.2416; Table 1).

Discussion

Biogeographical studies have greatly changed in the past few years. Traditional cladistic biogeography (Nelson & Platnick, 1981) based on general area cladograms derived from topological congruencies is giving way to an integrative historical biogeography

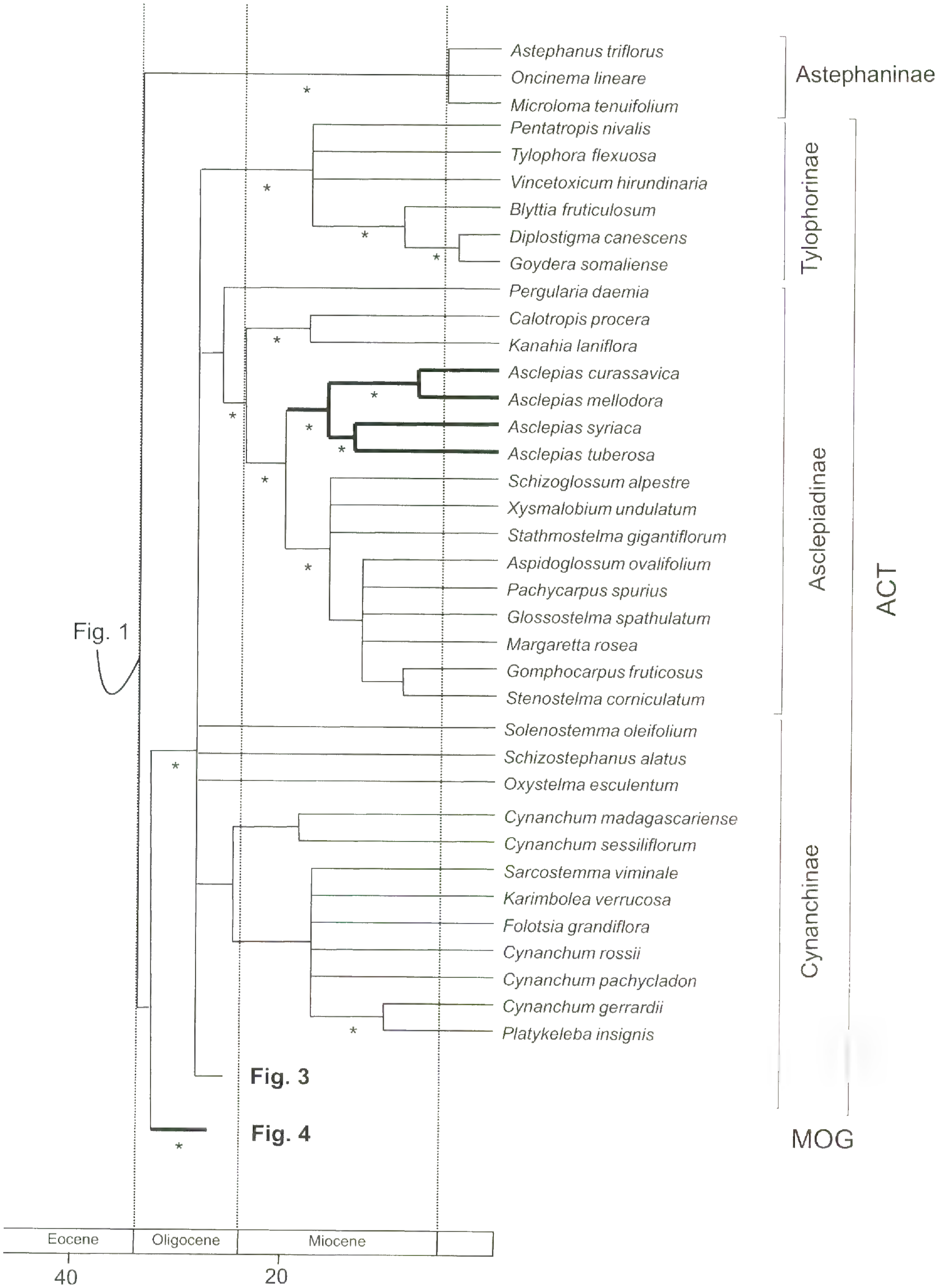

Fig. 1

Fig. 3

Fig. 4

Figure 2 (continued from Fig. 1). Upper portion of the chronogram in Figure 1 (Asclepiadeae). New World clades are in bold lines. Asterisks (*) identify clades with posterior probability greater than 94%.

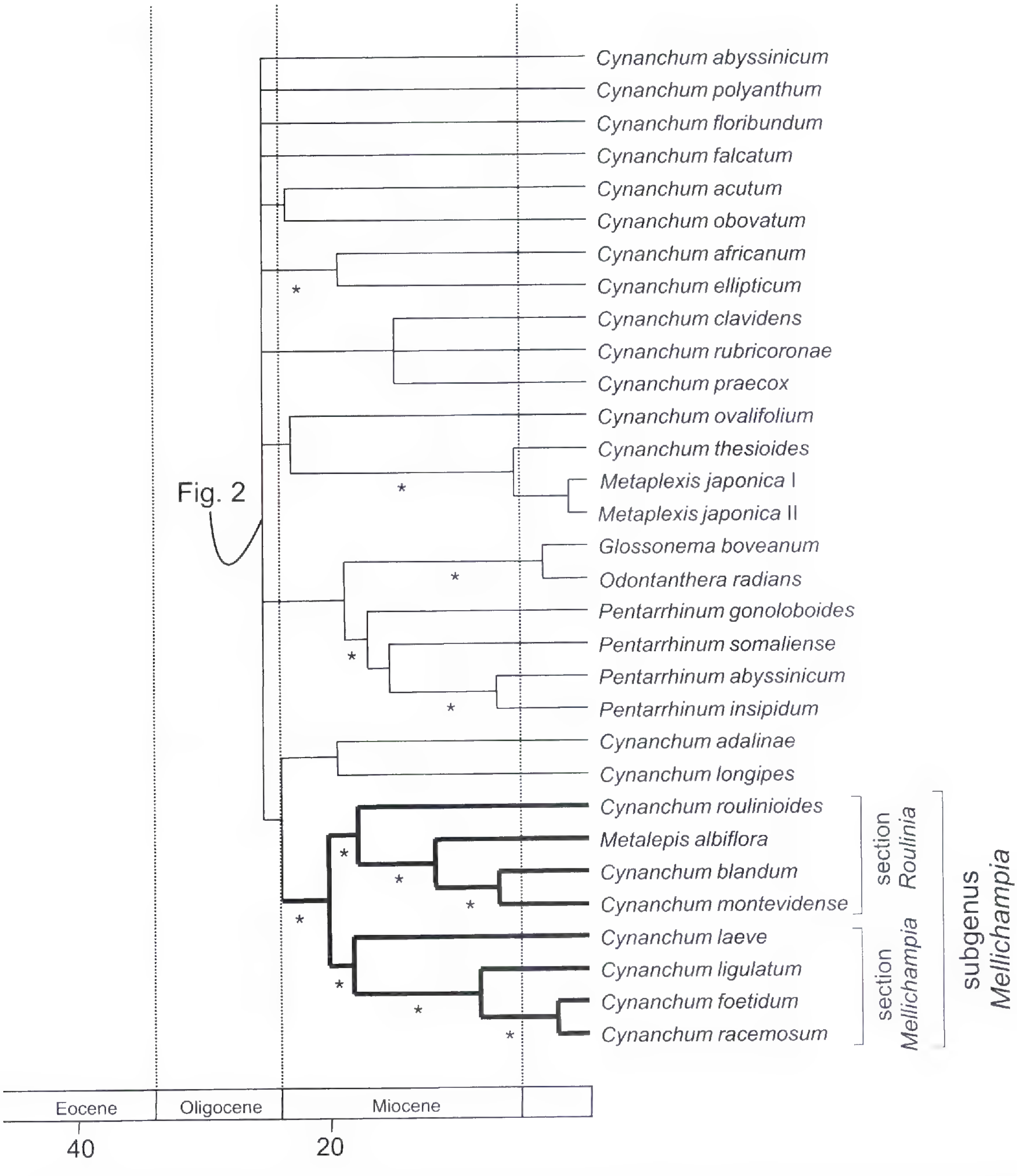

Fig. 2

Figure 3 (continued from Fig. 2). Upper portion of the chronogram in Figure 2 (part of Cynanchinae). New World clades are in bold lines. Asterisks (*) identify clades with posterior probability greater than 94%.

(Donoghue & Moore, 2003). Molecular phylogenetic data allow estimates of clade ages, making the association of patterns of dispersal and diversification to climate and geological events more realistic, while also demonstrating the temporal complexity of organism distribution by exposing pseudocongruencies (Riddle, 2005, e.g., similar topologies with different time frames). New evidence on the evolution of plants has presented difficulties in explaining the distribution of some groups. Ages estimated for the migration of several plants to the New World are not synchronic as would be expected in cases of single vicariant events, nor are they congruent with dates believed to allow intercontinental migration through land bridges (Pennington & Dick, 2004). These and many other examples have brought back long-distance dispersal as a feasible explanation in the scenario of biogeography (Queiroz, 2005).

Based on the data available and calibration assumed, the Asclepiadoideae originated at most in

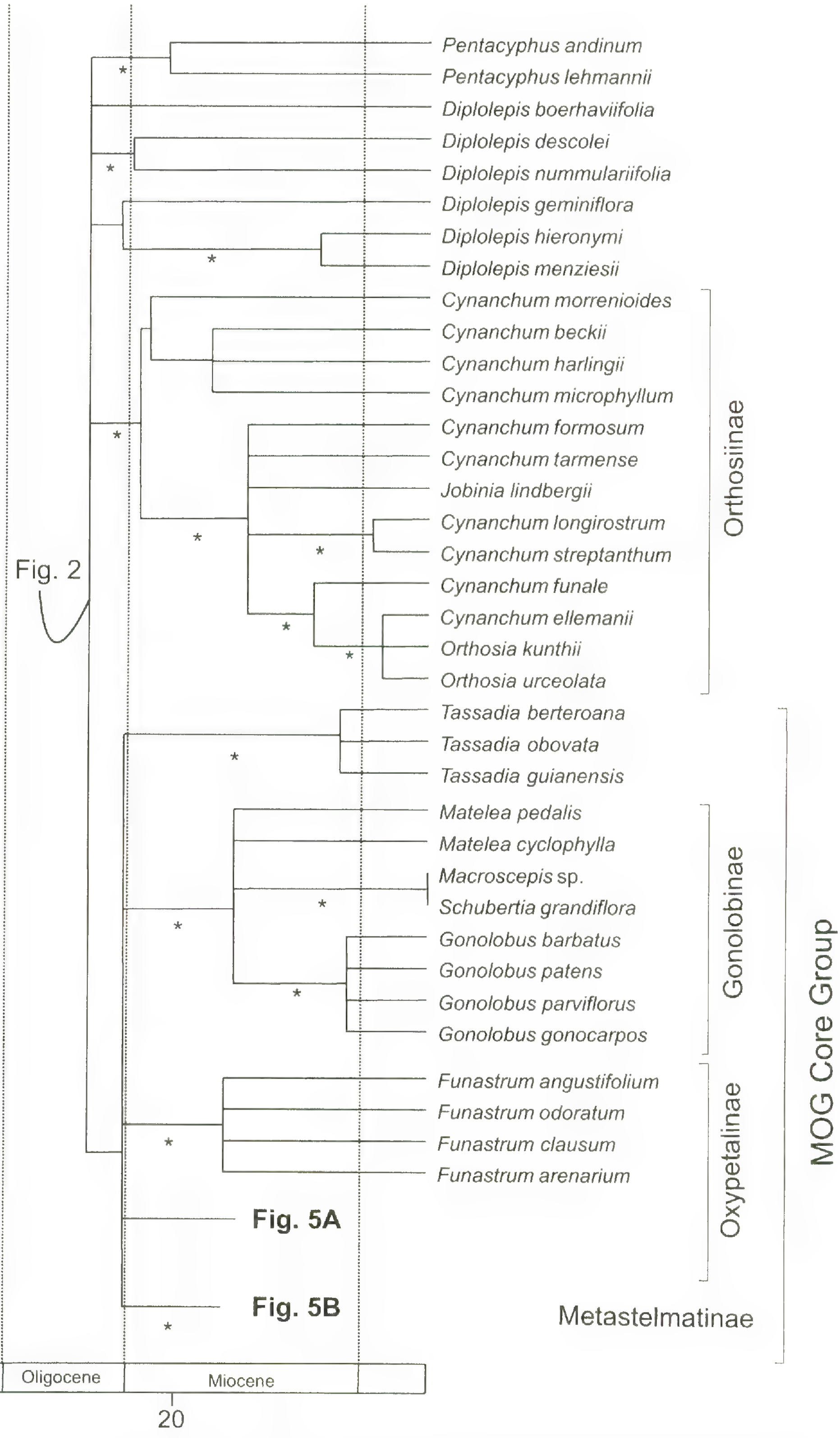

Figure 4 (continued from Fig. 2). Upper portion of the chronogram in Figure 2 (MOG). Asterisks (*) identify clades with posterior probability greater than 94%.

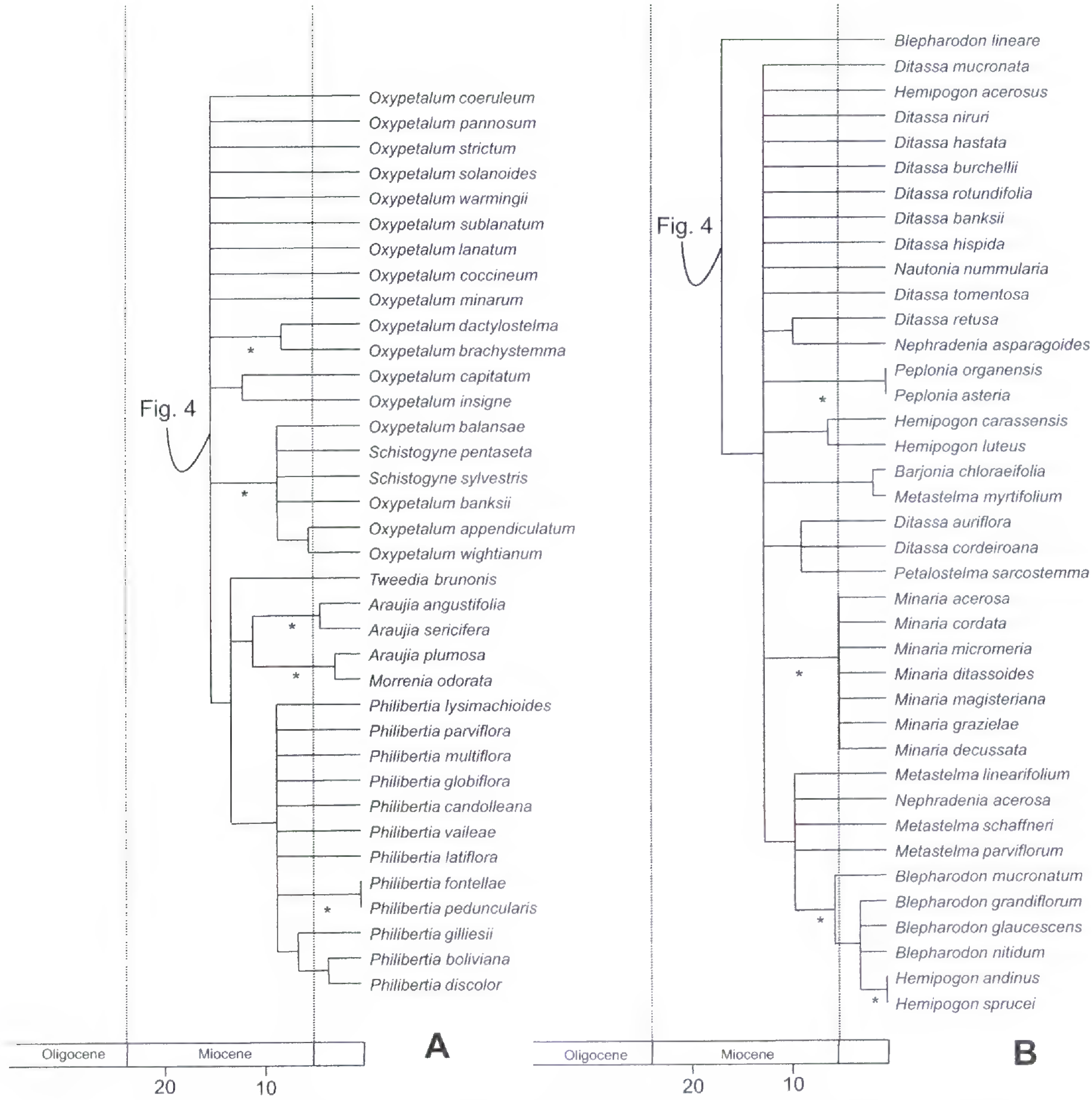

Figure 5 (continued from Fig. 4). Upper portion of the chronogram in Figure 4. —A. Oxypetalinae core group. —B. Metastelmatinae. Asterisks (*) identify clades with posterior probability greater than 94%.

the Late Eocene; fossil references of Asclepiadoideae in the Paleocene (e.g., LaMotte, 1952), therefore, must be treated with caution. Biogeographical events in the subfamily were not under the influence of Cretaceous continental drift that affected major floras before the Late Cretaceous (Raven & Axelrod, 1974a, b), and long-distance dispersals must be considered to explain intercontinental disjunctions between New and Old Worlds Asclepiadoideae. To determine the direction of dispersals in the discussion below, we usually assumed that if a derived clade A from an area X is nested in a grade composed of terminals from area Y, jump dispersal from Y to X is more likely. Directional asymmetry for long-distance dispersal, however, may make this assumption less simplistic (Cook & Crisp, 2005).

ORIGIN AND DIVERSIFICATION OF THE NEW WORLD ASCLEPIADOIDEAE

The Asclepiadoideae colonized the New World at four different times, suggesting independent dispersals from the Old World rather than any kind of vicariant event. The clade with Metastelmatinae, Oxypetalinae, and Gonolobinae (MOG, see introduction) was the first to arrive, at 32 Ma (Fig. 2); three other invasions were more recent, with *Cynanchum* at around 24 Ma (Fig. 3), *Asclepias* 20 Ma (Fig. 2), and *Marsdenia* 16 Ma (Fig. 1). Between 32 and 16 Ma, South America was a continental island (Raven & Axelrod, 1974a, b). In contrast, North America was connected to eastern Asia through the Bering Strait, a route probably permeable for temperate taxa until

Table 1. Number of species, age, and rates of speciation for New World Asclepiadoideae clades.

Clade	Species no. (n)[1]	Age (t)[2]	Rate of speciation[3]
MOG	**766**	**32**	**0.2075**
Pentacyphus	3	27	0.0407
Diplolepis	6	27	0.0664
Orthosiinae	77	27	0.1609
MOG core group	**680**	**27**	**0.2416**
Tassadia	24	24	0.1324
Gonolobinae	280	24	0.2348
Funastrum	17	24	0.1010
Other Oxypetalinae	177	24	0.2157
Blepharodon	2	16	0.0433
Other Metastelmatinae	180	16	0.3245
Asclepias	**125**	**20**	**0.2414**
Cynanchum subg. Mellichampia	**23**	**24**	**0.1306**
American *Marsdenia*	**70**	**16**	**0.2655**

[1] estimates.
[2] million years; based on stem group.
[3] speciation events per million years; birth-and-death model and extinction considered negligible: ln(n)/t.

the end of the Oligocene, but possibly later (Tiffney, 1985a).

MOG is a predominantly South American clade that comprises three quarters of New World Asclepiadoideae. It is characterized by a basal grade composed of the small South American genera *Pentacyphus* and *Diplolepis*, followed by the predominantly South American Orthosiinae (Liede-Schumann et al., 2005). This suggests that the first New World Asclepiadoideae arrived in South America. This colonization during the Oligocene must be explained by a transoceanic dispersal from Africa, the likely birthplace of the Asclepiadoideae. It is possible that the African Walvis Ridge and the South American Rio Grande Rise were above sea at this time, reducing the distance between the two continents (Renner, 2004), and long-distance dispersals between Africa and South America have been proposed to explain the origin of a substantial proportion of the Neotropical flora (Pennington & Dick, 2004).

The MOG core group arose at the end of Oligocene (Fig. 4). There is no consistent resolution for the relationship among the subtribes (Rapini et al., 2006), although the Metastelmatinae probably diverged first and the Oxypetalinae and Gonolobinae form a clade including *Tassadia* (Liede-Schumann et al., 2005).

The Metastelmatinae are well distributed along the Neotropics but reach higher diversity in the mountainous regions of the northern South America and central Brazil and in the Caribbean islands. This subtribe emerged in the Late Oligocene (24 Ma; Fig. 4) and radiated in the mid-Miocene (12 Ma; Fig. 5B). *Blepharodon* s. str., with two species restricted to central South America, was the first genus to diverge from the group, followed by a South American grade composed of *Minaria* and *Barjonia* Decne.–*Nephradenia* Decne. clade (Liede-Schumann et al., 2005; Rapini et al., 2006). *Minaria* includes 19 species, most of them restricted to small areas of the Espinhaço mountain range in Minas Gerais, Brazil (Konno et al., 2006). The genus arose at 12 Ma but did not radiate until 4.7 Ma, in the Tertiary–Quartenary boundary (Fig. 5B), denoting a relatively recent diversification in Brazilian shields. The *Barjonia–Nephradenia* clade comprises around a dozen species dispersed in central South America. The rest of Mestastelmatinae are divided into a few genera, the largest ones being *Ditassa* R. Br. and *Metastelma* R. Br. *Ditassa* is more diverse eastward of the tropical Andes, except in the Amazonian region, and *Metastelma* is predominantly distributed in the Caribbean islands (Liede & Meve, 2004). Their circumscription is still open, as well as the position of the smaller genera, which depends on further resolution of relationships in Metastelmatinae core group. The subtribe is therefore composed of a South American grade, in which the Central American species nest. Most species inhabit disturbed and/or open vegetation, which may have favored wind dispersal from South America to the Caribbean islands and their secondary diversification there.

The Oxypetalinae are most diverse in central South America, with the number of species greatly decreasing northward. The disproportionate distribution of Oxypetalinae in the Neotropics suggests that a primary diversification of the subtribe occurred between 15 –30 S, in central South America, during the mid-Miocene (Fig. 5A). *Funastrum* was the first genus to diverge in the evolution of the subtribe (Liede-Schumann et al., 2005; Rapini et al., 2006).

Arising in South America, *Funastrum* dispersed northward, becoming more diverse in Central and North America at 16 Ma (Fig. 4). Apart from *Funastrum*, the Oxypetalinae are divided into two main clades (Rapini et al., 2006), one predominantly in southeastern South America comprising *Oxypetalum* R. Br. (including *Schistogyne* Hook. & Arn.), and the other predominantly in southwestern South America comprising *Tweedia* Hook. & Arn., *Araujia* Brot.–*Morrenia* Lindl., and *Philibertia* Kunth. *Oxypetalum* is the largest genus in the subtribe, with most species occurring in northern Argentina and southern and southeastern Brazil, but *O. cordifolium* (Vent.) Schltr. can reach as far as Mexico and Cuba. Like *Funastrum*, *Oxypetalum* radiated at 16 Ma, having a second event of diversification at around 8 Ma, coinciding with *Philibertia* diversification (Fig. 5A).

The Gonolobinae are widespread in the Neotropics, presenting higher diversity from northern South America to Mexico. The circumscriptions of *Matelea* Aubl. and *Gonolobus* Michx., the two largest genera of Gonolobinae, are still open and the recognition of several genera in the subtribe depends on them. The subtribe emerged between the Oligocene and Miocene, presenting two episodes of diversification—an initial one at 15 Ma, and a second event in *Gonolobus* at 6.4 Ma (Fig. 4). From an initial diversification of the MOG core group in central South America, Gonolobinae would have dispersed west- and northward, radiating in northern South America and Central America, but not in the Caribbean islands, and ultimately reaching North America, where the subtribe is also well represented.

Tassadia is phylogenetically closer to Oxypetalinae and/or Gonolobinae than to Metastelmatinae (Liede-Schumann et al., 2005). The genus would have emerged during the initial diversification of the MOG core group but radiated only in the Late Miocene (Fig. 4). Most *Tassadia* species are concentrated in northern South America (Fontella-Pereira, 1977), with over half of them occurring in Venezuela. The most widespread species, *T. obovata*, reaches Santa Catarina in southern Brazil and Nicaragua to the North, and is the only species of *Tassadia* to cross the Isthmus of Panama into Central America.

The Cynanchinae are not yet well resolved in the ACT (Asclepiadinae, Cynanchinae, Tylophorinae) clade (Rapini et al., 2003), and *Cynanchum* (sensu Liede & Täuber, 2002), though not contradicted, was not detected with *trnL-F* (Rapini et al., 2003). The New World species, however, form a well-supported clade, the subgenus *Mellichampia* (Fig. 3; Liede & Kunze, 2002; Liede & Täuber, 2002; Rapini et al., 2003). This group is poorly diversified in the whole Neotropics, and according to phylogenetic studies with molecular data (e.g., Liede & Kunze, 2002), it can be roughly divided into North (section *Mellichampia* Sundell) and South (section *Roulinia* Sundell) American sections. However, *C. racemosum* (Jacq.) Jacq. is widespread in Central America, reaching northern South America, and *C. monteridense* Spreng. is widespread in South America, reaching Panama. Derivation of the two species, *C. racemosum* in section *Mellichampia* and *C. montevidense* in section *Roulinia* (Fig. 3), suggests that the occurrence of these species in South and Central America, respectively, is the result of relatively recent biotic expansions. Unlike the other three New World Asclepiadoideae lineages, the subgenus presented a slow, gradual accumulation of species since its origin in the Late Oligocene.

The Asclepiadinae arose in the Oligocene, reaching the New World in the Miocene (Fig. 2). *Asclepias* is the only American genus of the subtribe. Different from MOG, it is a predominantly North American group that is able to survive in colder regions. The number of species decreases southward, almost disappearing in the tropics but becoming more diverse in subtropical South America. *Asclepias* is sister to the African Asclepiadinae and is probably characterized by a basal grade of North American species, in which the South American clade is nested (Rapini et al., 2003; Goyder et al., 2007, this issue). The Asclepiadinae probably arrived in the New World through North America, dispersing to Central and South America. The North Atlantic passageway between Africa and North America persisted only until the Eocene (Tiffney, 1985b), and the increasingly cooler temperatures during the Late Tertiary made the connection between eastern Asia and western North America questionable (Tiffney, 1985a). As in Asclepiadinae, however, many plant disjunctions between Asia and North America seem to be more recent than 30 Ma, suggesting that plants have a high capacity for dispersing over long distances and establishing founder populations and that they also have higher extinction rates when compared to animals (Donoghue & Smith, 2004; Pennington & Dick, 2004). In this context, the Asclepiadinae have been able to disperse from Asia to North America, the predominant direction for plants, at around 20 Ma, through the Bering Strait during one of the several warm intervals that took place between Early Oligocene and mid-Miocene (Graham, 1999). The assumed Asian sister group of *Asclepias*, however, is hypothesized as extinct.

Although the South American *Asclepias* form a small, morphologically uniform group easily recognized by their white flowers, phylogenetically, the

colored flower *A. curassavica* is probably closer to them than to the North American species. If the species is originally North American, *Asclepias* would have dispersed to South America at 7.5 Ma. At this time, the Panamanian Isthmus was not yet established but probably supported a step-stone dispersal between the two continents. Alternatively, if *A. curassavica* is native to South America, this dispersal would be pushed to between 16 and 7.5 Ma, with the explanation for the dispersal to South America through long-distance dispersal or, at best, a step-stone migration through available inter-island passages (Coney, 1982). After arriving in South America, *Asclepias* has either diversified on the southern continent or evenly colonized the continent during cooler periods, and it was subject to extinction in tropical areas when the climate became warmer.

The Marsdenieae presumably arose in the Oligocene, arriving in the New World during the mid-Miocene and radiating in the Late Miocene (Fig. 1). *Marsdenia* is the only genus of Marsdenieae in the New World, inhabiting a wide range of vegetations from moist Amazonian forest in northern South America to dry caatinga in northeastern Brazil. The origin of *Marsdenia* in the New World is unclear, particularly because the group is poorly sampled and relationships to the American clade are unresolved. It is unlikely that they arrived in the New World via North America during the Miocene because the Bering Strait is believed to have been closed for megathermal plants since the Eocene (Tiffney, 1985a). The most plausible explanation is that *Marsdenia* arrived in South America by long-distance dispersal and shared the common northward dispersal route of New World lianas (Gentry, 1982), from tropical South America to Mesoamerica, probably through the Isthmus of Panama.

PATTERNS OF DIVERSIFICATION IN THE NEW WORLD ASCLEPIADOIDEAE

In Asclepiadoideae, invasions of the New World occurred in different periods of the Tertiary, involving long-distance dispersals from the Old World. The current diversity of the MOG clade, the oldest and most diverse Neotropical lineage of Asclepiadoideae, is concentrated in particular clades of the core group with higher rates of diversification, reflecting successive radiations, mainly during the Late Oligocene and mid-Miocene (Figs. 4, 5). The Late Oligocene is marked by a relatively warm temperature followed by the short-term Miocene glaciation, and the mid-Miocene marks the climatic optimum, between 15 and 16 Ma (Zachos et al., 2001).

The pattern of diversification detected in the MOG core group differs from that observed for elements of the Amazonian flora. Several species in *Funastrum*, *Oxypetalum*, and *Ditassa* are probably older than 10 Ma. On the other hand, evidence from phylogenetic studies in *Inga* Mill. (Richardson et al., 2001), a predominantly Amazonian species-rich genus of trees, shows speciation events concentrated in the past 10 Ma, with many species as young as 2 Ma or less. In some aspects, the pattern found in MOG diversification appears to be similar to that detected in Neotropical seasonally dry forest plants, whose lineages are also marked by multiple events of rapid speciation from the mid-Miocene to the Pliocene, resulting in a mosaic of ancient and recent species (Pennington et al., 2004).

The American *Cynanchum* were characterized by slow, gradual accumulation of species, allowing good resolution of relationships among them (Fig. 3). The relatively high rate of diversification of American *Marsdenia*, its recent dispersal to the Neotropics, and the unresolved relationship among species sampled here suggest that the group diversified by radiation. Based on the rate of diversification in *Asclepias*, similar to that of MOG core group, a diversification by radiations might also be assumed.

Somewhat synchronic radiations in MOG and ACT during the Late Oligocene, as well as among subtribes of MOG core group during the mid- and Late Miocene, suggest an important influence of global environmental factors in the Asclepiadoideae diversification. Intrinsic factors, however, have also played an essential role in their diversification, and they are probably the main reason why some lineages (e.g., *Pentacyphus*, *Diplolepis*, and *Blepharodon* s. str. in MOG, and *Cynanchum* subg. *Mellichampia* in Cynanchinae) have been subject to a remarkably low rate of diversification (unless extinction was extensive in these groups).

Together, these data suggest that there is not a single key element responsible for the diversification of Asclepiadoideae in the New World. Neither intrinsic innovations nor global extrinsic factors alone can explain this diversity. The imbalance of diversification in the clades of MOG seems to follow the general pattern observed in angiosperms as a whole, a complex process driven by interactive effects of biological traits and environmental factors (Davies et al., 2004). Apparently, most diversity, particularly in the MOG core clade, was the result of pulses of radiation allowed by intrinsic properties of lineages but motivated by environmental factors.

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Appendix 1. List of taxa and GenBank accession numbers. Asclepiad sequences were previously published in Rapini et al. (2003) and Liede-Schuman et al. (2005), as well as Liede and Taüber (2002), Meve and Liede (2004), and Rapini et al. (2006); classification and organization follows Endress and Bruyns (2000), Rapini et al. (2003), and Liede-Schumann et al. (2005).

Taxa	Intron *trnL*	Intergenic spacer *trnL-F*
LOGANIACEAE		
Strychnos tomentosa Benth.	AF214301	AF214147
GELSEMINACEAE		
Gelsemium sempervirens (L.) J. St.-Hil.	AF102428	AF159696
APOCYNACEAE		
Apocynoideae		
Apocynum androsaemifolium L.	AF214308	AF214154
Asclepiadoideae, Asclepiadeae		
Asclepiadeae, Astephaninae		
Astephanus triflorus R. Br	AJ410188	AJ410189
Microloma tenuifolium K. Schum.	AJ410221	AJ410222
Oncinema lineare (L. f.) Bullock	AJ410230	AJ410231
Asclepiadeae, ACT (Asclepiadinae, Cynanchinae, Tylophorinae)		
Asclepiadeae, Asclepiadinae		
Asclepias curassavica L.	AY163664	AY163664
Asclepias mellodora A. St.-Hil.	AY163665	AY163665
Asclepias syriaca L.	AF214311	AJ410180
Asclepias tuberosa L.	AF214312	AF214158
Aspidoglossum oralifolium (Schltr.) Kupicha	AY163666	AY163666
Calotropis procera (Aiton) W. T. Aiton	AF214324	AF214170
Glossostelma spathulatum (K. Schum.) Bullock	AY163686	AY163686
Gomphocarpus fruticosus (L.) W. T. Aiton	AY163687	AY163687
Kanahia laniflora (Forssk.) R. Br.	AY163695	AY163695
Margaretta rosea Oliv.	AY163696	AY163696
Pachycarpus spurius (N. E. Br.) Bullock	AY163716	AY163716
Pergularia daemia (Forssk.) Chiov.	AJ290892	AJ290893
Schizoglossum alpestre K. Schum.	AY163718	AY163718
Stathmostelma gigantiflorum K. Schum.	AY163721	AY163721
Stenostelma corniculatum (E. Mey.) Bullock	AY163722	AY163722
Xysmalobium undulatum (L.) W. T. Aiton	AY163725	AY163725
Asclepiadeae, Cynanchinae		
Cynanchum abyssinicum Decne.	AJ428580	AJ428581
Cynanchum acutum L.	AJ428583	AJ428584
Cynanchum adalinae K. Schum.	AJ428586	AJ428587
Cynanchum africanum Hoffmanns.	AJ428589	AJ428590
Cynanchum blandum (Decne.) Sundell	AJ428604	AJ428605
Cynanchum clavidens N. E. Br.	AJ428610	AJ428611
Cynanchum ellipticum (Harv.) R. A. Dyer.	AJ290846	AJ290845
Cynanchum falcatum Hutch. & E. A. Bruce	AJ428631	AJ428632
Cynanchum floribundum R. Br.	AJ428634	AJ428635
Cynanchum foetidum (Cav.) Kunth	AJ428637	AJ428638
Cynanchum gerrardii (Harv.) Liede	AJ428646	AJ428647
Cynanchum laeve (Michx.) Pers.	AJ428652	AJ428653
Cynanchum ligulatum (Benth.) Woodson	AJ428658	AJ428659
Cynanchum longipes N. E. Br.	AJ428664	AJ428665
Cynanchum madagascariense K. Schum.	AJ428667	AJ428668
Cynanchum montevidense Spreng.	AJ290849	AJ290850
Cynanchum obovatum (Decne.) Choux.	AJ428802	AJ428803

Appendix 1. Continued.

Taxa	Intron *trnL*	Intergenic spacer *trnL-F*
Cynanchum ovalifolium Wight	AJ428697	AJ428698
Cynanchum pachycladon Choux	AJ428700	AJ428701
Cynanchum polyanthum (K. Schum.) K. Schum.	AJ428712	AJ428713
Cynanchum praecox Schltr. ex S. Moore	AJ428715	AJ428716
Cynanchum racemosum (Jacq.) Jacq.	AJ428721	AJ428722
Cynanchum rossii Rauh	AJ428730	AJ428731
Cynanchum roulinioides (E. Fourn.) Rapini	AJ428733	AJ428734
Cynanchum rubricoronae Liede	AJ428736	AJ428737
Cynanchum sessiliflorum (Decne.) Liede	AJ428742	AJ428743
Cynanchum thesioides (Freyn) K. Schum.	AJ428748	AJ428749
Folotsia grandiflora (Jum. & H. Perrier) Jum. & H. Perrier	AJ290855	AJ290856
Glossonema boveanum (Decne.) Decne.	AY163684	AY163685
Karimbolea verrucosa Desc.	AJ290880	AJ290879
Metalepis albiflora Urb.	AJ428775	AJ428776
Metaplexis japonica Makino (I)	AJ428811	AJ428812
Metaplexis japonica Makino (II, wrongly assigned to *Cynanchum auriculatum* Buch.-Ham. ex Wight in previous studies)	AJ410197	AJ410198
Odontanthera radians (Forssk.) D. V. Field	AJ428814	AJ428815
Pentarrhinum abyssinicum Decne.	AJ428817	AJ428818
Pentarrhinum gonoloboides (Schltr.) Liede	AJ428820	AJ428821
Pentarrhinum insipidum E. Mey.	AJ410233	AJ410234
Pentarrhinum somaliense (N. E. Br.) Liede	AJ428823	AJ428824
Platykeleba insignis N. E. Br.	AJ290907	AJ290906
Sarcostemma viminale (L.) R. Br.	AJ290913	AJ290912
Schizostephanus alatus Hochst. ex K. Schum.	AJ410248	AJ410249
Asclepiadeae, Tylophorinae		
Biondia henryi (Warb. ex Schltr. & Diels) Tsiang & P. T. Li	AJ410191	AJ410192
Blyttia fruticulosa (Decne.) D. V. Field	AJ410194	AJ410195
Diplostigma canescens K. Schum.	AJ410200	AJ410201
Goydera somaliensis Liede	AJ410209	AJ410210
Pentatropis nivalis (J. F. Gmel.) D. V. Field & J. R. I. Wood	AJ410239	AJ410240
Tylophora flexuosa R. Br.	AJ290916	AJ290917
Vincetoxicum hirundinaria Medik.	AJ410275	AJ410276
Unplaced Genera		
Oxystelma esculentum (L. f.) Sm.	AJ290885	AJ290887
Solenostemma oleifolium (Nect.) Bullock & E. A. Bruce ex Bullock	AJ428832	AJ428833
Asclepiadeae, MOG (Metastelmatinae, Oxypetalinae, and Gonolobinae, plus Orthosiinae)		
Basal Grade		
Diplolepis boerhaviifolia (Hook. & Arn.) Liede & Rapini	AJ428607	AJ428608
Diplolepis descolei (T. Mey.) Liede & Rapini	AJ699304	AJ699302
Diplolepis geminiflora (Decne.) Liede & Rapini	AJ410182	AJ410183
Diplolepis hieronymi (Lorentz) Liede & Rapini	AJ410212	AJ410213
Diplolepis menziesii Schult.	AJ699273	AJ699275
Diplolepis nummulariifolia (Hook. & Arn.) Liede & Rapini	AJ290852	AJ290851
Pentacyphus andinus (Ball.) Liede	AJ492150	AJ492151
Pentacyphus lehmannii (Schltr.) Liede	AJ290889	AJ290888
Asclepiadeae, Gonolobinae		
Gonolobus barbatus Kunth	AJ704261	AJ704263
Gonolobus gonocarpos (Walter) L. M. Perry	AJ704277	AJ704276
Gonolobus parviflorus Decne.	AY163689	AY163689
Gonolobus rostratus (Vahl) Schult.	AF214362	AF214208
Macroscepis Kunth, sp. indet.	AJ704268	AJ704267
Matelea cyclophylla (Standl.) Woodson	AJ704269	AJ704272
Matelea pedalis (E. Fourn.) Fontella & E. A. Schwarz	AY163699	AY163699
Schubertia grandiflora Mart.	AJ428826	AJ428827
Asclepiadeae, Metastelmatinae		
Barjonia chloraeifolia Decne.	AY163667	AY163667

Appendix 1. Continued.

Taxa	Intron *trnL*	Intergenic spacer *trnL-F*
Blepharodon glaucescens (Decne.) Fontella	AJ699289	AJ699291
Blepharodon grandiflorum Benth.	AJ290837	AJ290838
Blepharodon lineare (Decne.) Decne.	AY163668	AY163668
Blepharodon mucronatum Decne.	AJ290840	AJ290839
Blepharodon nitidum (Vell.) J. F. Macbr.	AY163669	AY163669
Ditassa auriflora Rapini	AJ704471	AJ704470
Ditassa banksii R. Br. ex Schult.	AY163674	AY163674
Ditassa burchellii Hook. & Arn.	AJ699296	AJ699295
Ditassa cordeiroana Fontella	AY163675	AY163676
Ditassa hastata Decne.	AJ704221	AJ704223
Ditassa hispida (Vell.) Fontella	AJ704478	AJ704480
Ditassa mucronata Mart.	AJ704259	AJ704278
Ditassa niruri Decne.	AJ428751	AJ428752
Ditassa retusa Mart.	AJ704283	AJ704282
Ditassa rotundifolia (Decne.) Baill. ex K. Schum.	AJ704284	AJ704286
Ditassa subtrivialis Griseb.	AJ428755	AJ428756
Ditassa tomentosa (Decne.) Fontella	AJ701484	AJ704486
Hemipogon acerosus Decne.	AJ701291	AJ704290
Hemipogon andinum Rusby	AJ701292	AJ704294
Hemipogon luteus E. Fourn.	AY163693	AY163693
Hemipogon sprucei E. Fourn.	AJ701299	AJ704298
Metastelma linearifolium A. Rich.	AJ428808	AJ428809
Metastelma myrtifolium Decne.	AJ704494	AJ704493
Metastelma sp. indet., aff. *parviflorum* R. Br.	AJ428778	AJ428779
Metastelma schaffneri A. Gray	AJ410215	AJ410216
Minaria acerosa (Mart.) T. U. P. Konno & Rapini	AJ699288	AJ699287
Minaria cordata (Turcz.) T. U. P. Konno & Rapini	AJ699297	AJ699299
Minaria decussata (Mart.) T. U. P. Konno & Rapini	AJ704220	AJ704219
Minaria ditassoides (Silveira) T. U. P. Konno & Rapini	AY163678	AY163678
Minaria grazielae (Fontella & Marquete) T. U. P. Konno & Rapini	AJ410203	AJ410204
Minaria magisteriana (Rapini) T. U. P. Konno & Rapini	AY163681	AY163681
Minaria micromeria (Decne.) T. U. P. Konno & Rapini	AJ704248	AJ704237
Nautonia nummularia Decne.	AJ410227	AJ410228
Nephradenia acerosa Decne.	AY163704	AY163705
Nephradenia asparagoides (Decn.) E. Fourn.	AY163706	AY163707
Peplonia asteria (Vell.) Fontella & E. A. Schwarz	AJ704300	AJ704302
Peplonia organensis (E. Fourn.) Fontella & Rapini	AY163688	AY163688
Petalostelma sarcostemma (Lillo) Liede & Meve	AJ428787	AJ428788
Asclepiadeae, Orthosiinae		
Cynanchum beckii Morillo	AJ704307	AJ704306
Cynanchum ellemannii Morillo	AJ428781	AJ428782
Cynanchum formosum N. E. Br.	AJ428640	AJ428641
Cynanchum funale Poir.	AY163703	AY163703
Cynanchum harlingii Morillo	AJ704308	AJ704310
Cynanchum longirostrum (K. Schum.) W. D. Stevens	AJ704315	AJ704314
Cynanchum microphyllum Kunth	AJ428682	AJ428683
Cynanchum morrenioides Goyder	AJ428685	AJ428686
Cynanchum streptanthum Malme	AJ704316	AJ704318
Cynanchum tarmense Schltr.	AJ428745	AJ428746
Jobinia lindbergii E. Fourn.	AY163694	AY163694
Orthosia kunthii Decne.	AJ428784	AJ428785
Orthosia urceolata E. Fourn.	AJ704323	AJ704325
Asclepiadeae, Oxypetalinae		
Araujia angustifolia Steud.	AJ704330	AJ704332
Araujia plumosa Schltr.	AJ704337	AJ704336
Araujia sericifera Brot.	AJ428793	AJ428794
Funastrum angustifolium (Pers.) Liede & Meve	AJ428760	AJ428761
Funastrum arenarium (Decne. ex Benth.) Liede	AJ290858	AJ290857

Appendix 1. Continued.

Taxa	Intron *trnL*	Intergenic spacer *trnL-F*
Funastrum clausum (Jacq.) Schltr.	AJ290861	AJ290862
Funastrum odoratum Schltr.	AJ290870	AJ290871
Morrenia odorata (Hook. & Arn.) Lindl.	AJ704345	AJ704344
Oxypetalum appendiculatum Mart.	AY163709	AY163709
Oxypetalum balansae Malme	AJ704346	AJ704348
Oxypetalum banksii R. Br. ex Schult.	AY163710	AY163710
Oxypetalum brachystemma Malme	AJ704353	AJ704352
Oxypetalum capitatum Mart.	AY163711	AY163711
Oxypetalum coccineum Griseb.	AJ704329	AJ704326
Oxypetalum coeruleum (D. Don ex Sweet) Decne.	AJ704354	AJ704356
Oxypetalum dactylostelma Goyder	AJ704338	AJ704339
Oxypetalum insigne (Decne.) Malme	AY163712	AY163712
Oxypetalum lanatum Decne.	AJ704507	AJ704508
Oxypetalum minarum E. Fourn.	AY163713	AY163713
Oxypetalum pannosum Decne.	AJ704513	AJ704514
Oxypetalum solanoides Hook. & Arn.	AJ704361	AJ704360
Oxypetalum strictum Mart.	AY163714	AY163714
Oxypetalum sublanatum Malme	AY163715	AY163715
Oxypetalum warmingii (E. Fourn.) Fontella & Marquete	AJ704519	AJ704520
Oxypetalum wightianum Hook. & Arn.	AJ704524	AJ704523
Philibertia boliviana (Baill.) Goyder	AJ704233	AJ704232
Philibertia candolleana (Hook. & Arn.) Goyder	AJ410176	AJ410177
Philibertia discolor (Schltr.) Goyder	AY163700	AY163700
Philibertia fontellae Goyder	AJ492153	AJ492154
Philibertia gilliesii Hook. & Arn.	AJ290895	AJ290894
Philibertia globiflora Goyder	AJ704234	AJ704236
Philibertia latiflora (Griseb.) Goyder	AJ704241	AJ704242
Philibertia lysimachioides (Wedd.) T. Mey.	AJ290901	AJ290900
Philibertia multiflora (T. Mey.) Goyder	AJ704243	AJ704245
Philibertia parviflora (Malme) Goyder	AJ410224	AJ410225
Philibertia peduncularis (Benth.) Goyder	AJ704251	AJ704250
Philibertia vaileae (Rusby) Liede	AJ290904	AJ290905
Schistogyne pentaseta Rusby	AJ704252	AJ704254
Schistogyne sylvestris Hook. & Arn.	AJ410245	AJ410246
Tweedia brunonis Hook. & Arn.	AJ704260	AJ704258
Asclepiadoideae, MOG Unplaced Genus		
Tassadia berteroana (Spreng.) W. D. Stevens	AJ428790	AJ428791
Tassadia guianensis Decne.	AJ699280	AJ699279
Tassadia obovata Decne.	AJ699281	AJ699283
Asclepiadoideae, Ceropegieae		
Anisotoma cordifolia Fenzl	AJ410017	AJ410018
Caralluma arachnoidea (P. R. O. Bally) M. G. Gilbert	AJ410038	AJ410039
Ceropegia juncea Roxb.	AJ428799	AJ428800
Ceropegia saxatilis Jum. & H. Perrier	AJ410041	AJ410042
Heterostemma cuspidatum Decne.	AJ574829	AJ574828
Leptadenia arborea (Forssk.) Schweinf.	AJ574833	AJ574834
Stapelia glanduliflora Mass.	AJ402128	AJ402151
Stapelia leendertziae N. E. Br.	AF214424	AF214270
Asclepiadoideae, Eustegieae		
Eustegia minuta (L. f.) N. E. Br.	AJ410206	AJ410207
Asclepiadoideae, Fockeeae		
Fockea edulis K. Schum.	AF214353	AF214199
Asclepiadoideae, Marsdenieae		
Cionura erecta Griseb.	AJ410173	AJ410174
Dischidia bengalensis Colebr.	AF214343	AF214189
Gymnema inodorum (Lour.) Decne.	AJ431750	AJ431751
Hoya australis R. Br. ex J. Traill	AF214367	AF214213

Appendix 1. Continued.

Taxa	Intron *trnL*	Intergenic spacer *trnL-F*
Marsdenia amorimii Morillo	AF214377	AF214223
Marsdenia gillespieae Morillo	AJ431756	AJ431757
Marsdenia macrophylla (Humb. & Bonpl. ex Schult.) E. Fourn.	AJ574821	AJ574822
Marsdenia megalantha Goyder & Morillo	AJ574836	AJ574835
Marsdenia rubicunda N. E. Br.	AJ574839	AJ574840
Marsdenia suberosa (E. Fourn.) Malme	AY163697	AY163697
Marsdenia tenacissima (Roxb.) Moon	AJ431759	AJ431760
Marsdenia verrucosa Decne.	AJ431762	AJ431763
Marsdenia zehntneri Fontella	AY163698	AY163698
Micholitzia obcordata N. E. Br.	AF214381	AF214227
Neoschumannia kamerunensis Schltr.	AJ410053	AJ410054
Telosma accedens (Blume) Backer	AJ431783	AJ431784
Telosma cordata Merr.	AF214280	AF102493
Periplocoideae		
Periploca graeca L.	AF102468	AF214241
Rauvolfioideae		
Plumeria alba L.	AF214408	AF214254
Rauvolfia serpentina (L.) Benth. ex Kurz	AF214415	AF214261
Secamonoideae		
Pervillaea tomentosa Decne.	AJ431768	AJ431769
Secamone alpinii Schult.	AJ428829	AJ428830
Secamone glaberrima K. Schum.	AF214420	AF214266

PHYLOGENETIC RELATIONSHIPS IN SUBTRIBE ASCLEPIADINAE (APOCYNACEAE: ASCLEPIADOIDEAE)[1]

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ABSTRACT

A first approximation of evolutionary relationships within the Asclepiadinae (Apocynaceae: Asclepiadoideae) is inferred from two DNA data sets, from the nuclear ITS region, and from plastid *trnT-L* and *trnL-F* spacers and *trnL* intron. Both the subtribe as a whole and the phylogenetic radiation of largely herbaceous genera around *Asclepias* are monophyletic and well supported. While resolution within the *Asclepias* complex is limited, the lineages identified demonstrate that current generic delimitation is unsatisfactory. *Asclepias* should be either restricted to New World members of the subtribe or expanded to encompass the entire radiation. In the African species, it is shown that, although the corona is taxonomically important for recognizing species, it can be misleading as an indicator of phylogenetic relationship. Vegetative similarities often prove a more reliable guide, but more detailed studies are needed to refine these lineages and to detect morphological characters to aid in their practical recognition.

Key words: Apocynaceae, Asclepiadaceae, Asclepiadinae, phylogenetics, ITS, *trnT-L*, *trnL-F*, *trnL*.

Generic delimitation has long been problematic in the Asclepiadinae (Apocynaceae: Asclepiadoideae) sensu Liede (1997). Brown (1902: 299) commented in the *Flora of Tropical Africa*: "Undoubtedly *Xysmalobium* R. Br., *Asclepias* L. and *Schizoglossum* E. Mey. are but artificial divisions of one natural genus, since they cannot be separated by characters that do not break down at some point." Nevertheless, he went on to assign species to the three genera on the basis of the form of the corona—cucullate in *Asclepias*, dorsally flattened in *Schizoglossum*, and thickened or laterally compressed in *Xysmalobium*. Such an artificial arrangement separated apparently allied species, such as *A. lisianthoides* (Decne.) N. E. Br., *S. carsonii* (N. E. Br.) N. E. Br., and *X. ceciliae* N. E. Br., all currently referred to *Glossostelma* Schltr. (Goyder, 1995). Subsequent morphological studies have proposed the use of non-coronal characters to reunite some of these natural groups. Bullock (1952, 1953a, b, 1955, 1956, 1957, 1961) started the process with a number of groups in tropical Africa but never articulated his reasoning beyond a statement that *Asclepias* should refer only to New World species and that he proposed to "exclude *Asclepias* Linn. from Africa, except as an adventive, and to divide further the species hitherto assigned to it into other genera" (1952: 406). Some of Bullock's generic concepts have been developed by later workers—*Aspidoglossum* E. Mey., *Miraglossum* Kupicha, and *Schizoglossum* (Kupicha, 1984); *Aspidonepsis* Nicholas & Goyder (Nicholas & Goyder, 1992); *Glossostelma* (Goyder, 1995); *Gomphocarpus* R. Br. (Goyder, 2001b; Goyder & Nicholas, 2001); *Margaretta* Oliv. (Goyder, 2005); *Pachycarpus* E. Mey. (Smith, 1988; Goyder, 1998a); *Stathmostelma* K. Schum. (Goyder, 1998b); and *Trachycalymma* (K. Schum.) Bullock (Goyder, 2001a). In southern Africa, Bullock's unpublished views on *Xysmalobium* sensu N. E. Br. (1902), as deduced from annotations of herbarium collections housed at K, formed the basis of a postgraduate thesis (Langley, 1980), but the generic changes proposed therein were never formally published. Likewise, unpublished theses by Nicholas (1982, 1999) provide a framework for the *Asclepias* complex in southern Africa, but a satisfactory synthesis of tropical and southern African taxa is still lacking. The present contribution is designed firstly to test the monophyly of the *Asclepias* generic complex, and secondly to see what lineages can be detected within this assemblage.

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Material and Methods

Taxa

Sixty-five species in 21 out of the 24 recognized Asclepiadinae genera and one undescribed genus constitute the ingroup (Table 1). Two accessions from different localities were analyzed in each of five species (*Asclepias crispa* P. J. Bergius, *A. gibba* (E. Mey.) Schltr., *Aspidoglossum heterophyllum* E. Mey., *Gomphocarpus physocarpus*, and *Kanahia laniflora* (Forssk.) R. Br.). In *Asclepias*, the only genus represented in both the Old World and the New World, 10 Old World species and six New World species were included. *Marsdenia sylvestris* (Retz.) P. I. Forst. (Marsdenieae) was used as the most distant outgroup. As closer outgroups, species were selected from the closely related subtribes of the Asclepiadeae, the Tylophorinae, and Cynanchinae, including *Glossonema* Decne. and *Odontanthera* Wight. Exemplars from the American Metastelminae–Gonolobinae–Oxypetalinae assemblage, sister to the Asclepiadinae–Cynanchinae–Tylophorinae clade (Rapini et al., 2003; Liede-Schumann et al., 2005; Goyder, 2006), were not included, because studies by Liede (2001) and Liede and Täuber (2002) had shown that sequences from this taxonomic group added numerous long indels but presented little additional information as to the directionality of site changes. Instead, taxa previously found not to fit in any of the recognized subtribes were included: *Eustegia minuta* (L. f.) N. E. Br. (Rapini et al., 2003); *Calciphila galgalensis* (Liede) Liede & Meve (Liede & Kunze, 2002; Liede-Schumann & Meve, 2006); *Oxystelma bornouense* R. Br. and *O. esculentum* (L. f.) Schult. (Liede & Täuber, 2000); and *Solenostemma arghel* (Delile) Hayne (Liede et al., 2002a—as *S. oleifolium* (Nectoux) Bullock & E. A. Bruce).

DNA extraction and PCR

DNA was isolated from fresh or dried leaf tissue using the CTAB protocols (Doyle & Doyle, 1987). Polymerase chain reaction (PCR) primers and protocol for the plastid *trnT-L* and *trnL-F* spacers (primers "a," "b" and "e," "f," respectively) and the *trnL* intron (primers "c" and "d") correspond exactly to Taberlet et al. (1991). Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer (Applied Biosystems, Foster City, California, U.S.A.). Of the 80 accessions, 65 have been sequenced for this study; the remaining 15 were previously deposited at European Molecular Biology Laboratory (EMBL; Table 1). The *trnL* intron and *trnL-F* spacer of *Asclepias syriaca* L. and *A. tuberosa* L. were taken from EMBL, and we were able to supplement the missing partial sequences (*trnT-L* spacer and ITS) from our own material for *A. syriaca*, but not for *A. tuberosa*.

The entire nuclear internal transcribed spacer region (ITS) including 5.8S of ribosomal DNA (rDNA) was amplified using the flanking primers ITS4 and ITS5 following a modified protocol based on Baldwin (1992) and described in Meve and Liede (2001). ITS sequences of 68 species were obtained for the present study, with one sequence studied previously. An interpretable ITS sequence could not be obtained for *Trachyealymma pseudofimbriatum* Goyder. Published or unpublished ITS sequences by the last author are available for all outgroup taxa except for *Oxystelma bornouense*. However, only those of *O. esculentum* and *Solenostemma arghel* could be aligned to the Asclepiadinae sequences, albeit with difficulties.

Data analysis

Sequences were pre-aligned with Sequence Navigator Version 1.0.1 (Perkin-Elmer, Inc., Waltham, Massachusetts, U.S.A.) and manually adjusted. Separate indel coding was not performed, because the number of phylogenetically informative indels was very low in the chloroplast DNA (cpDNA) data set, while the indels could not be coded unambiguously in the ITS data set. Both data sets were analyzed separately and in combination. Phylogenetic analysis and tests for clade support were performed, using PAUP version 4.0b8 (PPC) (Swofford, 1998). Phylogenies were generated using Fitch parsimony as implemented in PAUP* employing heuristic searches, with 1000 replicates, random stepwise addition, MULPARS off, and tree-bisection-reconnection (TBR) branch swapping. The resulting trees were then used as starting trees for a second round of search with MULPARS on. Our search strategy aimed at finding as many different islands of trees as possible. Sets of equally parsimonious trees recovered from each analysis were summarized by strict consensus. Decay indices (Bremer, 1988; Donoghue et al., 1992) and bootstrap values (Felsenstein, 1985) derived from 1000 replicates (maxtrees set to 100) were calculated as measures of support for individual clades. Decay analyses were performed with AutoDecay 4.0 (Eriksson, 1998) in combination with the reverse constraint option of PAUP*. A partition homogeneity test as implemented in PAUP* was conducted (10 addition sequence replicates) to test the congruence of the cpDNA and the ITS data sets.

Results

The sequence alignment is available from the authors and from TreeBASE (study accession number

Table 1. Voucher specimens and EMBL accession numbers for the taxa investigated. Sequences already published: A = Liede and Täuber (2000); B = Liede and Kunze (2002); C = Liede (2001); D = Liede et al. (2002a); E = Liede et al. (2002b); F = Meve and Liede (2001); and G = Potgieter and Albert (2001). ITS sequence numbers in square brackets refer to published sequences that could not be fitted into the ITS alignment.

Tribe, subtribe	Taxon	DNA voucher	*trnT-L* spacer	*trnL* intron	*trnL*-F spacer	ITS
Marsdenieae	*Marsdenia sylvestris* (Retz.) P. I. Forst.	Cameroon: Mokolo, *Meve 919* (UBT)	AJ402118 (F)	AJ402137 (F)	AJ402142 (F)	unpubl.
Asclepiadeae, unplaced	*Calciphila galgalensis* (Liede) Liede & Meve	Somalia: Bari, *Thulin & Warfa 6205* (UPS)	AJ492337 (B)	AJ492338 (B)	AJ492339 (B)	[AJ492756 (B)]
	Eustegia minuta (L. f.) N. E. Br.	South Africa: *Bruyns 4357* (K)	AJ410205 (C)	AJ410206 (C)	AJ410207 (C)	unpubl.
	Oxystelma bornouense R. Br.	Kenya: Tsavo East, *Liede & Newton 3210* (UBT)	AJ290883 (A)	AJ290882 (A)	AJ290881 (A)	—
	Oxystelma esculentum (L. f.) Schult.	Egypt: Elephantine Island, cult. Bayreuth ex *Shirley s.n.* (UBT)	AJ290884 (A)	AJ290885 (A)	AJ290887 (A)	AM396877
	Solenostemma arghel (Delile) Hayne	Egypt: *Aziz s.n.* (MO)	AJ428831 (D)	AJ428832 (D)	AJ428833 (D)	AM396878
Asclepiadeae, Cynanchinae	*Cynanchum ellipticum* (Harv.) R. A. Dyer	South Africa: Eastern Cape, *Liede 2933* (UBT)	AJ290845 (A)	AJ290846 (A)	AJ290847 (A)	[AJ320444 (E)]
	Glossonema boveanum (Decne.) Decne.	Kenya: Loyengalani, *Liede & Newton 3239* (ULM)	AJ428804 (D)	AJ428805 (D)	AJ428806 (D)	[AJ492803 (B)]
	Odontanthera radians (Forssk.) D. V. Field	Yemen: North, *Müller-Hohenstein & Deil 1967* (UBT)	AJ428813 (D)	AJ428814 (D)	AJ428815 (D)	[AJ492809 (B)]
Asclepiadeae, Tylophorinae	*Tylophora flexuosa* R. Br.	Philippines: Laguna, *Liede 3252* (UBT)	AJ290915 (A)	AJ290916 (A)	AJ190917 (A)	[AJ320460 (E)]
Asclepiadeae, Asclepiadinae	*Asclepias albens* (E. Mey.) Schltr.	South Africa: Eastern Cape, *Nicholas 2813* (UDW)	AM295719	AM295720	AM295721	AM396879
	Asclepias brevipes (Schltr.) Schltr.	South Africa: Free State, *Nicholas 2866* (UDW)	AM295724	AM295723	AM295722	AM396880
	Asclepias crispa P. J. Bergius, acc. 1	South Africa: Eastern Cape, *Nicholas 2814* (UDW)	AM295725	AM295726	AM295727	AM396881
	Asclepias crispa P. J. Bergius, acc. 2	South Africa: Eastern Cape, *Nicholas 2823* (UDW)	AM295730	AM295729	AM295728	AM396882
	Asclepias cucullata (Schltr.) Schltr.	South Africa: KwaZulu-Natal, *Nicholas 2842* (UDW)	AM295731	AM295732	AM295733	AM396883
	Asclepias curassavica L., acc. 1	Venezuela: Mérida, *Liede & Meve 3316* (UBT)	AM295734	—	—	AM396884
	Asclepias curassavica L., acc. 2	U.S.A.: *Potgieter & Cahilly 243* (NY)	—	AF102381 (G)	AF214156 (G)	—
	Asclepias fascicularis Decne.	U.S.A.: ex hort UC Davis	AM295735	AM295736	AM295737	AM396885

Table 1. Continued.

Tribe, subtribe	Taxon	DNA voucher	*trnT-L* spacer	*trnL* intron	*trnL*-F spacer	ITS
	Asclepias gibba (E. Mey.) Schltr., acc. 1	South Africa: Eastern Cape, *Nicholas 2824* (UDW)	AM295740	AM295739	AM295738	AM396886
	Asclepias gibba (E. Mey.) Schltr., acc. 2	South Africa: Eastern Cape, *Nicholas 2838* (UDW)	AM295741	AM295742	AM295743	AM396887
	Asclepias linaria Cav.	Mexico: Michoacán, *Conrad 9305* (UBT)	AM295746	AM295745	AM295744	AM396888
	Asclepias longifolia Michx.	U.S.A.: *Wyatt s.n.* (UGA)	AM295747	AM295748	AM295749	AM396889
	Asclepias praemorsa Schltr.	South Africa: Transkei, *Ollerton 214* (NU, spirit)	AM295718	AM295717	AM295716	AM396890
	Asclepias stellifera Schltr.	South Africa: KwaZulu-Natal, *Nicholas 2841* (UDW)	AM295713	AM295714	AM295715	AM396891
	Asclepias syriaca L.	ex hort. Münster, in cult. Münster (MSUN)	AJ410178 (C)	AJ410179 (C)	AJ410180 (C)	AM396892
	Asclepias tuberosa L.	U.S.A.: *Potgieter & Cahilly 242* (NY)	–	AF214312 (G)	AF141158 (G)	—
	Asclepias woodii (Schltr.) Schltr.	South Africa: KwaZulu-Natal, *Ollerton 255* (NU, spirit)	AM295712	AM295711	AM295710	AM396893
	Aspidoglossum connatum (N. E. Br.) Bullock	Tanzania: Nkansi District, *Bidgood et al. 2583* (K)	AM295700	AM295699	AM295698	AM396894
	Aspidoglossum delagoense (Schltr.) Kupicha	South Africa: KwaZulu-Natal, *Nicholas 2877* (UDW)	AM295707	AM295708	AM295709	AM396895
	Aspidoglossum heterophyllum E. Mey., acc. 1	South Africa: Western Cape, *Bruyns 6855* (K)	AM295701	AM295702	AM295703	AM396896
	Aspidoglossum heterophyllum E. Mey., acc. 2	South Africa: Eastern Cape, *Nicholas 2816* (UDW)	AM295706	AM295705	AM295704	AM396897
	Aspidonepsis diploglossa (Turcz.) Nicholas & Goyder	South Africa: KwaZulu-Natal, *Ollerton 228* (NU, spirit)	AM295695	AM295696	AM295697	AM396898
	Aspidonepsis flava (N. E. Br.) Nicholas & Goyder	South Africa: KwaZulu-Natal, *Ollerton 223* (NU, spirit)	AM295694	AM295693	AM295692	AM396899
	Calotropis procera (Aiton) W. T. Aiton	Gambia: *Huber s.n.* (UBT)	AJ428795 (D)	AJ428796 (D)	AJ428797 (D)	AM396900
	Cordylogyne globosa E. Mey.	South Africa: Mpumalanga, *Nicholas 2867* (UDW)	AM295691	AM295690	AM295689	AM396901
	Fanninia caloglossa Harv.	South Africa: *Ollerton 217* (NU, in spirit)	AM295686	AM295687	AM295688	AM396902
	Glossostelma ceciliae (N. E. Br.) Goyder	Tanzania: Ufipa District, *Goyder et al. 3834* (K)	AM295685	AM295769	AM295768	AM396903

Table 1. Continued.

Tribe, subtribe	Taxon	DNA voucher	*trnT-L* spacer	*trnL* intron	*trnL*-F spacer	ITS
	Gomphocarpus abyssinicus Decne.	Ethiopia: Shewa Region, *Liede & Meve 3520* (UBT)	AM295752	AM295751	AM295750	AM396904
	Gomphocarpus cancellatus (Burm. f.) Bruyns	South Africa: *Drewe 534* (K)	AM295765	AM295766	AM295767	AM396905
	Gomphocarpus fruticosus (L.) W. T. Aiton	South Africa: Eastern Cape, *Nicholas 2796* (UDW)	AM295764	AM295763	AM295762	AM396906
	Gomphocarpus glaucophyllus Schltr.	Zambia: Lusaka, *Strid 2266* (K)	AM295759	AM295760	AM295761	AM396907
	Gomphocarpus physocarpus E. Mey., acc. 1	South Africa: Eastern Cape, *Nicholas 2798* (UDW)	AM295758	AM295757	AM295756	AM396908
	Gomphocarpus physocarpus E. Mey., acc. 2	South Africa: Eastern Cape, *Nicholas 2829* (NH)	AJ290875 (A)	AJ290876 (A)	AJ290877 (A)	AJ320446 (E)
	Gomphocarpus tomentosus Burch.	South Africa: KwaZulu-Natal, *Nicholas 2878* (UDW)	AM295753	AM295754	AM295755	AM396872
	Kanahia laniflora (Forssk.) R. Br., acc. 1	Kenya: Tsavo East, *Liede & Newton 3211* (ULM)	AM295577	AM295578	AM295579	AM396873
	Kanahia laniflora (Forssk.) R. Br., acc. 2	Yemen: *Mangelsdorff 116* (UBT)	AM295582	AM295581	AM295580	AM396874
	Lagarinthus multicaulis E. Mey.	South Africa: Eastern Cape, *Nicholas 2836* (UDW)	AM295583	AM295584	AM295585	AM396875
	Margaretta rosea Oliv. subsp. *corallina* Goyder	Tanzania: Ufipa District, *Goyder et al. 3791* (K)	AM295588	AM295587	AM295586	AM396876
	Miraglossum verticillare (Schltr.) Kupicha	South Africa: KwaZulu-Natal, *Ollerton 244* (NU, spirit)	AM295589	AM295590	AM295591	AM396840
	Pachycarpus appendiculatus E. Mey.	South Africa: KwaZulu-Natal, *Nicholas 2871* (UDW)	AM295594	AM295593	AM295592	AM396841
	Pachycarpus asperifolius Meisn.	South Africa: Limpopo, *Nicholas 2858* (UDW)	AM295595	AM295596	AM295597	AM396842
	Pachycarpus concolor E. Mey.	South Africa: KwaZulu-Natal, *Nicholas 2856* (UDW)	AM295600	AM295599	AM295598	AM396843
	Pachycarpus coronarius E. Mey.	South Africa: Eastern Cape, *Nicholas 2812* (UDW)	AM295601	AM295602	AM295603	AM396844
	Pachycarpus dealbatus E. Mey.	South Africa: KwaZulu-Natal, *Nicholas 2849* (UDW)	AM295606	AM295605	AM295604	AM396845
	Pachycarpus goetzei (K. Schum.) Bullock	Tanzania: Njombe District, *Bidgood et al. 2106* (K)	AM295607	AM295608	AM295609	AM396846

Table 1. Continued.

Tribe, subtribe	Taxon	DNA voucher	*trnT-L* spacer	*trnL* intron	*trnL*-F spacer	ITS
	Pachycarpus grantii (Oliv.) Bullock subsp. *marroninus* Goyder	Tanzania: Njombe District, *Goyder et al. 3886* (K)	AM295612	AM295611	AM295610	AM396847
	Pachycarpus lineolatus (Decne.) Bullock	Tanzania: Ufipa District, *Goyder et al. 3779* (K)	AM295613	AM295614	AM295615	AM396848
	Pachycarpus natalensis N. E. Br.	South Africa: KwaZulu-Natal, *Ollerton 254* (NU, spirit)	AM295618	AM295617	AM295616	AM396849
	Pachycarpus reflectens E. Mey.	South Africa: Eastern Cape, *Nicholas 2801* (UDW)	AM295619	AM295620	AM295621	AM396850
	Pergularia daemia (Forssk.) Chiov.	Tanzania: Arusha, *Masinde 888* (in cult. Bayreuth)	AJ290891 (A)	AJ290892 (A)	AJ290893 (A)	AM396851
	Schizoglossum atropurpureum E. Mey.	South Africa: Eastern Cape, *Ollerton 216* (NU, spirit)	AM295624	AM295623	AM295622	AM396852
	Schizoglossum cordifolium E. Mey.	South Africa: *Ward s.n.* (UDW)	AM295625	AM295626	AM295627	AM396853
	Schizoglossum eustegioides (E. Mey.) Druce	South Africa: North Western Province, *Nicholas 2865* (UDW)	AM295630	AM295629	AM295628	AM396854
	Schizoglossum filiforme (L. f.) Druce	South Africa: KwaZulu-Natal, *Nicholas 2850* (UDW)	AM295631	AM295632	AM295633	AM396855
	Stathmostelma diversifolium Goyder	Kenya: Narok, *Liede & Newton 3221* (ULM)	AM295636	AM295635	AM295634	AM396856
	Stathmostelma pauciflorum (Klotzsch) K. Schum.	Zimbabwe: Karoi, *Nicholas 2860* (UDW)	AM295637	AM295638	AM295639	AM396857
	Stathmostelma verdickii De Wild.	Tanzania: Ufipa District, *Bidgood et al. 2647* (K)	AM295642	AM295641	AM295640	AM396858
	Stenostelma corniculatum (E. Mey.) Bullock	South Africa: Mpumalanga, *Balkwill 10908* (K)	AM295643	AM295644	AM295645	AM396859
	Trachycalymma buchwaldii (Schltr. & K. Schum.) Goyder	Tanzania: Iringa Distr., *Goyder et al. 3924* (K)	AM295648	AM295647	AM295646	AM396860
	Trachycalymma foliosum (K. Schum.) Goyder	Tanzania: Ufipa District, *Goldblatt et al. 8100* (K)	AM295684	AM295649	AM295650	AM396861
	Trachycalymma pseudofimbriatum Goyder	Ethiopia: Sidamo, *Haugen 1503* (K)	AM295653	AM295652	AM295651	—
	Woodia mucronata (Thunb.) N. E. Br.	South Africa: Eastern Cape, *Nicholas 2809* (UDW)	AM295654	AM295655	AM295656	AM396862
	Xysmalobium fraternum N. E. Br.	Tanzania: Sumbawanga District, *Bidgood et al. 2390* (K)	AM295659	AM295658	AM295657	AM396863

Table 1. Continued.

Tribe, subtribe	Taxon	DNA voucher	*trnT-L* spacer	*trnL* intron	*trnL*-F spacer	ITS
	Xysmalobium gerrardii Scott-Elliott	South Africa: KwaZulu-Natal, *Ollerton 251* (NU, spirit)	AM295660	AM295661	AM295662	AM396864
	Xysmalobium heudelotianum Decne.	Tanzania: Ufipa District, *Goyder et al. 3830* (K)	AM295665	AM295664	AM295663	AM396865
	Xysmalobium involucratum (E. Mey.) Decne.	South Africa: Eastern Cape, *Nicholas 2802* (UDW)	AM295666	AM295667	AM295668	AM396866
	Xysmalobium kaessneri S. Moore	Tanzania: Mbeya District, *Lovett et al. 3980* (K)	AM295671	AM295670	AM295669	AM396867
	Xysmalobium parviflorum Harv. ex Scott-Elliott	South Africa: KwaZulu-Natal, *Nicholas 2840* (UDW)	AM295672	AM295673	AM295674	AM396868
	Xysmalobium tysonianum (Schltr.) N. E. Br.	South Africa: KwaZulu-Natal, *Ollerton 233* (NU, spirit)	AM295677	AM295676	AM295675	AM396869
	Xysmalobium undulatum (L.) W. T. Aiton	South Africa: Eastern Cape, *Nicholas 2830* (UDW)	AM295678	AM295679	AM295680	AM396870
	Gen. indet., aff. *Asclepias*	Tanzania: Njombe District, *Goyder et al. 3895* (K)	AM295683	AM295682	AM295681	AM396871

Table 2. Summary statistics of the data sets and of the trees resulting from parsimony analysis. CI = consistency index; RI = retention index; RC = rescaled consistency index.

	cpDNA	ITS	Combined
Parsimony informative characters	153	208	277
Unknown data cells	222 (*trnT-L* spacer) 50 (*trnL* intron) 131 (*trnL-F* spacer)	28	
Missing (partial) sequences	*Asclepias tuberosa* (*trnT-L* spacer)	*Asclepias tuberosa* *Trachycalymma pseudofimbriatum* *Oxystelma esculentum*	
Outgroup	*Marsdenia sylvestris* *Eustegia minuta* *Calciphila galgalensis* *Cynanchum ellipticum* *Glossonema boveanum* *Odontanthera radians* *Tylophora flexuosa* *Oxystelma bornouense* *Oxystelma esculentum* *Solenostemma arghel*	*Oxystelma esculentum* *Solenostemma arghel*	*Oxystelma esculentum* *Solenostemma arghel*
No. of trees (limited by computer capacity)	56,100	56,500	62,000
No. of steps	262	650	778
CI	0.71	0.5	0.52
RI	0.81	0.5	0.66
RC	0.57	0.32	0.34

= S1600, matrix accession numbers = M2879 (cpDNA), M2880 (ITS), M2881 (combined); Sanderson et al., 1994) and comprises 80 taxa and 2847 characters (1088 characters in the *trnT-L* spacer (primers a–b), 530 characters in the *trnL*-intron (primers c–d), 429 characters in the *trnL-F* spacer (primers e–f), and 800 characters for ITS). Details of the data sets, as well as statistics of the trees resulting from parsimony analysis, are listed in Table 2.

The partition homogeneity test shows no significant discordance between the cpDNA and the ITS data sets ($P = 0.09$), and the retrieved clade structure in the cpDNA data set agrees largely with the one retrieved from the ITS data set, despite the poor resolution of the former.

The analyses reveal that the Asclepiadinae as presently circumscribed are monophyletic. The small Old World genera *Pergularia* L. (*P. daemia* (Forssk.) Chiov.), *Calotropis* R. Br. (*C. procera* (Aiton) W. T. Aiton), and *Kanahia* R. Br. (*K. laniflora* (Forssk.) R. Br.) are sister to the remaining members of the subtribe, which themselves form a well-supported clade, referred to here as the *Asclepias* generic complex. Several smaller clades were detected, but overall resolution is limited. The genera in this complex will need to be recircumscribed following our results.

Discussion

The core group of principally herbaceous genera around *Asclepias* contains most of the species diversity within the Asclepiadinae. This group is both monophyletic and well supported, resolving into two clades in the ITS (not shown) and combined analyses (Fig. 1). One clade consists of taxa found only in the New World (*A. linaria* Cav., *A. curassavica* L., *A. fascicularis* Decne., *A. longifolia* Michx., and *A. syriaca* L.); the other consists solely of Old World taxa. The *trnT-L* data set reveals no overall resolution into New or Old World lineages, but several minor Old World clades and one minor New World clade are retrieved (not shown). *Asclepias*, the oldest generic name in the complex (1753), must be applied either to the whole assemblage or, if other genera are recognized, to just the New World clade, as this contains *A. syriaca*, its type species. This result is consistent with Bullock's (1952) assertion that *Asclepias* should be restricted to New World taxa. Within the African radiation, a number of clades were detected, which are well supported in one or more of the analyses.

A clade comprising *Xysmalobium heudelotianum* Decne., *X. fraternum* N. E. Br., *X. kaessneri* S. Moore, *Stathmostelma verdickii* De Wild. (a species excluded

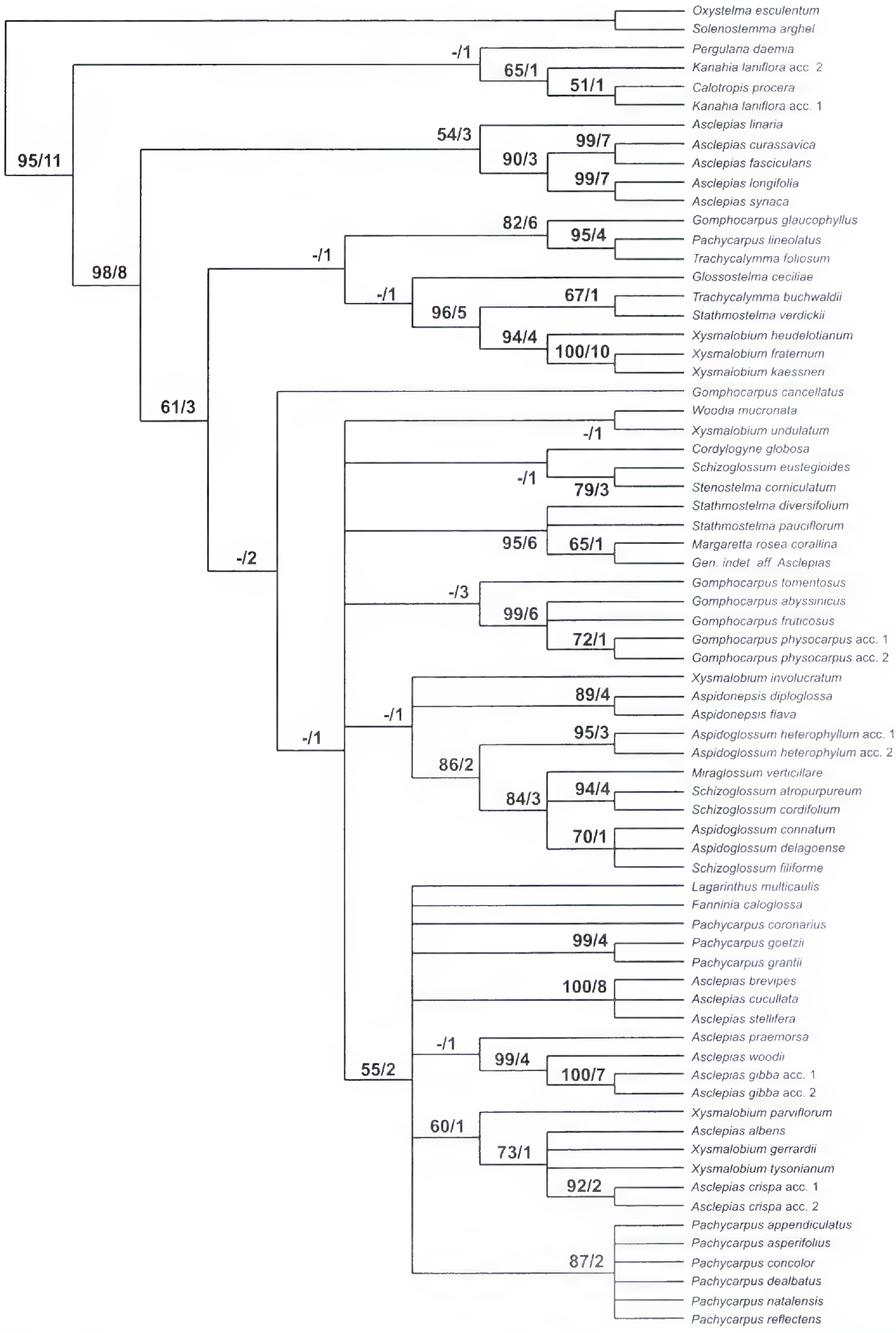

Figure 1. Strict consensus of 62,000 trees resulting from analysis of the combined data set (l = 778 steps, excluding uninformative characters, CI = 0.52, excluding uninformative characters, RI = 0.66, RC = 0.34). Numbers mapped on the tree indicate bootstrap percentages above 50% and decay values.

from *Stathmostelma* by Goyder (1998b) who suggested an affinity with *Gomphocarpus longissimus* K. Schum.), and *Trachycalymma buchwaldii* (Schltr. & K. Schum.) Goyder was detected in all analyses. These species are found in tropical African wooded savannas at moderate to high altitude. A second group of *Xysmalobium* species (*X. parviflorum* Harv. ex Scott-Elliott, *X. gerrardii* Scott-Elliott, and *X. tysonianum* (Schltr.) N. E. Br.) forms a clade with a group of broad-leaved *Asclepias* (*A. albens* (E. Mey.) Schltr. and *A. crispa*) with moderate support (60%) in all analyses. These species share a similar habit with many decumbent stems arising from a large woody tuber and have dense umbelliform pedunculate inflorescences. They occur in open grassland habitats in southeastern parts of tropical and subtropical Africa. The position of the apparently closely allied taxon, *Lagarinthus multicaulis* E. Mey. (Langley, 1980; = *A. multicaulis* (E. Mey.) Schltr. non *A. multicaulis* Vell.), is unresolved, however. *Xysmalobium*, clearly, is polyphyletic as currently circumscribed (Langley, 1980), and the type of the genus, *X. undulatum* (L.) W. T. Aiton, occurs in yet another clade separate from other exemplars of the genus.

Asclepias woodii (Schltr.) Schltr. links with *A. gibba* in ITS and the combined analyses with strong support (99%). This is surprising, because they do not share obvious similarities of habit and vegetative or floral morphology (Brown, 1908). *Asclepias gibba* is much branched from the base, while *A. woodii* is an erect, single-stemmed herb; the corona of *A. gibba* terminates in a long drawn-out apical tooth much taller than the staminal column, while that of *A. woodii* is shorter and lacks the terminal appendage.

The *Schizoglossum/Aspidoglossum/Miraglossum* complex of Kupicha (1984) is identified in all analyses (*A. heterophyllum*, *A. connatum* (N. E. Br.) Bullock, *A. delagoense* (Schltr.) Kupicha, *M. verticillare* (Schltr.) Kupicha, *S. atropurpureum* E. Mey., *S. cordifolium* E. Mey., and *S. filiforme* (L. f.) Druce; bootstrap 86% in the combined analysis). Kupicha (1984) noted the similarities of *S. filiforme* to species of *Aspidoglossum* but excluded it from the latter genus because of its pedunculate inflorescences and differing corona morphology. The results here link it to *A. connatum* and *A. delagoense*. *Schizoglossum eustegioides* (E. Mey.) Druce was excluded from the complex altogether by Kupicha (1984), who suggested its affinities might lie with *Stenostelma* Schltr.; ITS and the combined analyses support this suggestion, but there is no resolution with *trnT-L*.

A small group of narrow-leaved *Asclepias* species in southern Africa—*A. brevipes* (Schltr.) Schltr., *A. cucullata* (Schltr.) Schltr., and *A. stellifera* Schltr.—occurs in all analyses.

A group of short-lived *Gomphocarpus* species with narrow leaves (*G. abyssinicus* Decne., *G. fruticosus* (L.) W. T. Aiton, and *G. physocarpus* E. Mey.) forms a well-supported clade (bootstrap 99%) with ITS and the combined analyses but is not resolved with *trnT-L*. Neither *G. cancellatus* (Burm. f.) Bruyns nor *G. glaucophyllus* Schltr., both broad-leaved species, form part of this core *Gomphocarpus* lineage. *Gomphocarpus cancellatus* is the only species of the generic complex with a predominantly winter-rainfall distribution in southern Africa and has some anomalous morphological features, such as the combination of broad leaves with perennial rather than annual stems and the more robust processes on the follicle. *Gomphocarpus glaucophyllus* appears in a predominantly tropical clade sister to *Pachycarpus lineolatus* (Decne.) Bullock and *Trachycalymma foliosum* (K. Schum.) Goyder. *Gomphocarpus glaucophyllus* is found in burnt grasslands and savanna woodlands of south tropical Africa, and its broad glaucous leaves are borne on annual shoots from a perennial woody rootstock. In contrast, the narrow-leaved species of *Gomphocarpus* occur in open disturbed habitats in temperate regions of southern Africa and are shrubby in habit, but are probably short-lived and generally lack thickened or tuberous roots.

Stathmostelma diversifolium Goyder, *S. pauciflorum* (Klotzsch) K. Schum., *Margaretta rosea* Oliv. subsp. *corallina* Goyder, and the undescribed taxon, all from seasonally burned montane habitats in tropical Africa, also form a well-supported clade with ITS and the combined analysis but are unresolved with the chloroplast data set. Goyder (2005) pointed to similarities in corona morphology between *Margaretta* and *Stathmostelma*. The undescribed taxon is one of several from the highlands of southern Tanzania and northern Malawi that the first author has found difficult to assign to the genera currently recognized.

Finally, a group of southern African *Pachycarpus* species (*P. appendiculatus* E. Mey., *P. asperifolius* Meissn., *P. concolor* E. Mey., *P. dealbatus* E. Mey., *P. natalensis* N. E. Br., and *P. reflectens* E. Mey.) is detected in *trnT-L* and the combined analyses. Two of these (*P. concolor* and *P. dealbatus*) form a minor clade in ITS, as do two of the tropical species (*P. goetzei* (K. Schum.) Bullock and *P. grantii* (Oliv.) Bullock subsp. *marroninus* Goyder). However, *P. coronarius* E. Mey. remains in an unresolved position in the combined analysis, and *P. lineolatus*, a widely distributed tropical species, links somewhat unexpectedly to the south tropical African species *Gomphocarpus glaucophyllus*, as already indicated.

In the exemplars above, vegetative features seem a more reliable guide to evolutionary relationships than do the traditional floral characters. In particular,

there has been a switch between the *Xysmalobium*-type fleshy corona and *Asclepias*- or *Gomphocarpus*-type cucullate corona both in tropical and southern African clades. *Margaretta* and *Stathmostelma* sensu Goyder (1998b) have much more showy and brightly colored flowers than the small white flowers of the undescribed taxon but are similar vegetatively with erect annual stems arising from a carrot-shaped tuber.

The delimitation of *Asclepias* could be restricted to species from the Americas, and the Old World clade subdivided into genera with redefined limits. This would require a more intensively sampled survey and the use of additional genomes. Some of these lineages might correspond to the tropical group with erect, fusiform follicles (e.g., *Xysmalobium heudelotianum*); a more narrowly defined *Gomphocarpus*; an expanded *Margaretta* containing *Stathmostelma* and some tropical taxa currently unassigned to genus; *Schizoglossum* including *Miraglossum* and *Aspidoglossum* but excluding some anomalous taxa; and the broad-leaved group of *Xysmalobium* and *Asclepias* from southern African grasslands.

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ASCLEPIADOIDEAE (APOCYNACEAE) FROM SOUTHEASTERN BRAZIL. I. THE GENUS *OXYPETALUM* FROM RIO DE JANEIRO STATE, BRAZIL[1]

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ABSTRACT

Most genera of the subfamily Asclepiadoideae (i.e., 29 genera) can be found in the southeastern Brazilian region. Among these, the most representative is *Oxypetalum* R. Br., with 71 taxa (including specific and infraspecific). In Rio de Janeiro State, there are 25 taxa of *Oxypetalum* that are climbers, with the exception of *O. patulum* E. Fourn., which is a suberect shrub and decumbent. Most taxa from Rio de Janeiro occur in secondary Atlantic Forest, but four are from sandy coastal plain (restinga) vegetation, namely *O. alpinum* (Vell.) Fontella & E. A. Schwarz var. *alpinum*, *O. pachyglossum* Decne., *O. banksii* Schult. subsp. *banksii*, and *O. banksii* subsp. *corymbiferum* (E. Fourn.) Fontella & C. Valente. The latter species is restricted to restingas. Some taxa occur in high-altitude grasslands, namely *O. costae* Occhioni, *O. lanatum* Decne ex E. Fourn., *O. patulum* E. Fourn., *O. sublanatum* Malme, *O. insigne* (Decne.) Malme var. *insigne*, *O. insigne* var. *glabrum* (Decne.) Fontella & E. A. Schwarz, *O. insigne* var. *glaziovii* (E. Fourn.) Fontella & E. A. Schwarz, and *O. regnellii* (Malme) Malme. Four species and one subspecies are endemic to the state of Rio de Janeiro: *O. costae*, *O. glaziovianum* Loes., *O. lutescens* E. Fourn., *O. schottii* E. Fourn., and *O. banksii* subsp. *corymbiferum*. Two species are cited here as new records for the state of Rio de Janeiro: *O. molle* Hook. & Arn. and *O. pannosum* Decne. var. *pannosum*. The following taxa are proposed as new synonyms: *O. sobralii* Occhioni (= *O. schottii*), *O. villosum* E. Fourn. (= *O. pachyglossum* Decne.), and *O. mourai* Hoehne (= *O. glaziovianum* Loes.). *Oxypetalum appendiculatum* Mart., *O. molle*, *O. pannosum* var. *pannosum*, *O. sublanatum*, *O. wightianum* Hook. & Arn., and *O. banksii* subsp. *banksii* are widely distributed, the first five reaching Paraguay and Argentina, while *O. banksii* subsp. *banksii* is limited to Brazil (from Alagoas to Rio Grande do Sul). Lectotypifications were made for the following species: *Calostigma guilleminianum* Decne., *Cystostemma glandulosum* Silveira, *Oxypetalum glaziovianum* Loes., *O. lanatum*, *O. mourai*, *O. pachyglossum*, *O. regnellii*, *O. schottii*, *O. selloanum* E. Fourn., and *O. sublanatum*. Types are designated for *Schizostemma* Decne. and *Pachyglossum* Decne. as synonyms of *Oxypetalum*.

Key words: Apocynaceae, Asclepiadoideae, Brazil, *Oxypetalum*, Rio de Janeiro.

Rio de Janeiro State is located in southeastern Brazil. It borders on the states of Minas Gerais to the north and northwest, São Paulo to the west, Espírito Santo to the northeast, and the Atlantic Ocean to the south, east, southeast, and southwest. It has an area of 43,909.7 km^2 and a coastline of 636 km. It belongs to the "Mata Atlântica" biome (Atlantic Forest), which is one of Brazil's largest forest formations both in area and in species diversity, second only to Amazônia (Paula, 2004). According to Morellato and Haddad (2000: 786), "the year 2000 marked 500 years of massive destruction for the Brazilian Atlantic Forest, as a consequence of the colonization of Brazil. Today, the Atlantic Forest is restricted to ca. 98.800 km^2 of remnants, or 7.6 percent of its original extension," and is one of the 25 biodiversity hotspots recognized in the world.

The Atlantic Forest has an estimated plant diversity of about 20,000 species, and about 6000 of these are considered endemic. Biome in Rio de Janeiro State has the following formations: rainforest, rainforest with *Araucaria* Juss., seasonal forest, restinga, mangrove, and high-altitude grasslands. It is considered to be well protected in Rio de Janeiro, where there are about 42 conservation units, including national and state parks, biological reserves, ecological stations, and environmental protection areas, forming 28% of the total remnant area (Centro de Informações e Dados do Rio de Janeiro, 1995). Significant to the study of *Oxypetalum*

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R. Br. in Rio de Janeiro are the Itatiaia National Park, Serra dos Órgãos National Park, Tijuca National Park, and Restinga de Jurubatiba National Park.

These national parks play an important role in the preservation of *Oxypetalum*. Of the 25 taxa found in Rio de Janeiro, 13 are from Itatiaia National Park, nine are from Serra dos Órgãos National Park, five are from Tijuca National Park, and three are from Restinga de Jurubatiba National Park. Species of this genus, such as *O. banksii* Schult. subsp. *banksii*, occur at sea level in the restinga but are also found at high altitudes in Itatiaia National Park. In the 20th century, the spectacular flora of Rio de Janeiro encouraged researchers from the Rio de Janeiro National Museum and the Rio de Janeiro Botanical Garden Research Institute (those with the largest herbaria) to collect more intensely. Especially noteworthy collections were made by the following researchers, listed with the areas they worked in: A. C. Brade from Itatiaia and Macaé (1933–1940), P. Campos Porto from Itatiaia and Serra dos Órgãos (1918, 1925, 1934), E. Pereira from Itatiaia and Serra dos Órgãos (1943–1944), and J. G. Kuhlmann from Serra dos Órgãos (1940). These voluminous collections made possible the publication of several regional treatments of well-known Brazilian botanists, such as Barroso (1957), Brade (1957), Rizzini (1957), Pereira (1965), Albuquerque (1968), Barroso et al. (1974), and more recently, Marques et al. (1997), with other family treatments, such as Fromm-Trinta (1997) and Coelho (1997). Given the high species diversity observed in Rio de Janeiro and the large number of specimens held in local herbaria, taxonomists from this state have been collaborating toward the Programa Flora do Rio de Janeiro.

Material and Methods

This study is based on the examination of ca. 576 herbarium specimens from B, BHCB, BM, BOTU, CAMB, CEN, CEPEC, CTES, CVRD, ESA, ESAL, F, FCQ, G, GFJP, GUA, HB, HBR, HRB, HRCB, HUEFS, IBGE, ICB, ICN, INPA, K, M, MBM, NY, OUPR, OXF, P, PAMG, R, RB, RBR, RFA, RUSU, UB, UFG, S, SP, SPF, TER, US, W, WU. Field work was carried out in 1978 and from 2001 to 2002. The pollinaria were fixed on temporary slides with 50% water and glycerin. Measurements were made with an Olympus BH2 optical microscope.

Taxonomy

Oxypetalum R. Br., Asclepiadeae 30. 1810, nom. cons. TYPE: *Oxypetalum banksii* R. Br. ex Schult.

Gothofreda Vent., Choix Pl. 36, t. 60. 1808. TYPE: *Gothofreda cordifolia* Vent.

Calostigma Decne., Ann. Sci. Nat., Bot., ser. 2, 9: 343. 1838. TYPE: *Calostigma insigne* Decne.

Schizostemma Decne., Ann. Sci. Nat., Bot., ser. 2, 9: 344. 1838. TYPE, designated here: *Schizostemma parviflora* Decne., Ann. Sci. Nat., Bot., ser. 2, 9: 345. 1838. *Oxypetalum parviflorum* (Decne.) Decne., Prodr. (DC.) 8: 587. 1844. *Gothofreda parviflora* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. lit. 1891.

Pachyglossum Decne., Ann. Sci. Nat., Bot., ser. 2, 9: 345. 1838. TYPE, designated here: *Oxypetalum pachyglossum* Decne., Prod. (DC.) 8: 585. 1844.

Bustelma E. Fourn., Fl. Bras. 6(4): 287. 1885. TYPE: *Bustelma warmingii* E. Fourn., Fl. Bras. 6(4): 288. 1885. TYPE: Brazil. Minas Gerais: Lagoa Santa, 28 Mar. 1864, *J. E. B. Warming 1862* (lectotype, designated by Fontella-Pereira & Marquete, 1971: 4, C!). [= *Oxypetalum warmingii* (E. Fourn.) Fontella & Marquete, 1971.]

Twining vine, erect, rarely decumbent; stem and branches usually pubescent to lanate, rarely glabrous or glabrescent; latex white. Leaves opposite, petiolate or ± sessile, glabrous to lanate; petiole adaxially with (0 to)1 to 5(to 7) colleters at blade base; blades ovate to oblong-lanceolate, usually base cordate and apex acuminate, veining brochidodromous or camptobrochidodromous. Inflorescences 1 per node, extra-axillary, sciadioidal, cymoid to corymbiform, dichasially sciadioidal-umbelliform and dichasially sciadioidal-corymbiform, usually pedunculate, bract linear to lanceolate, pedicels short or elongate. Perianth glabrous, glabrescent, or with trichomes nonglandular, uniseriate, pluricellular, smooth, or verrucose; calyx lobes basally fused, abaxially glabrous or with trichomes (1- to 10-celled) longer than trichomes of the corolla, adaxially usually glabrous, with 1 or 9 colleters below each sinus within; corolla rotate to campanulate, white, green, yellow, brown, red, or purple, lobes usually twisted, reflexed, spreading or erect, glabrous to glabrescent or densely covered with verrucose or smooth trichomes (1- to 7-celled); corona white, green, brown, yellow, purple, red, or vinaceous, fused to both the base of the staminal column and the lower part of the corolla tube, lobes simple, tooth adaxially present or absent; gynostegium sessile, ± sessile, or shortly stipitate; anthers winged, wings extending along the entire length of the anther, anther wings of adjacent anthers parallel to each other, usually longer than dorsum, with connective appendage of several shapes; corpusculum thick and linear to ± cylindrical or laminar in frontal view, usually without a membranaceous appendage at apex; caudicles horizontal, usually with teeth lateral, curved and free or descending to oblique with tooth indistinct and included, short or long-pedicellate; pollinia pendulous, or descending to oblique, longer or shorter than corpusculum, rarely equal; style-head white, purple, violet, dark red, or

vinaceous, usually elongated-conical and bifid, rarely depressed-conical to conical, usually exserted. Follicles 1 or 2 per flower, usually pedicellate and fusiform, usually smooth, glabrous to lanate; seeds verrucose and comose.

Several botanists have published important papers on *Oxypetalum* in Brazil, including Fournier (1885), Malme (1900, 1927), Hoehne (1916), Occhioni (1956), and Rapini et al. (2001). Goyder (2004), with his synopsis of the genus *Philibertia* Kunth, published a key to the genera most closely allied to *Oxypetalum*: these are *Widgrenia* Malme, *Schistogyne* Hook. & Arn., *Philibertia* Kunth, *Tweedia* Hook. & Arn., *Araujia* Brot., and *Morrenia* Lindl. A similar key was published by Krapovickas and Cáceres-Moral (1990).

Oxypetalum is found in the Neotropics and in subtropical regions stretching from Mexico to Argentina, with ca. 130 species (Rapini et al., 2003). In Brazil, there are about 110 known taxa. In Rio de Janeiro, these occur in low-montane rainforest, montane rainforest, high-altitude grasslands, and restinga, but in other states they also occur in gallery forest, rainforest with *Araucaria*, secondary forest, open secondary vegetation, seasonal forest, disturbed seasonal forest cerradão, cerrado-cerradão, cerrado, campo, and campo rupestre.

Lectotypifications of *Schizostemma* Decne. and *Pachyglossum* Decne. are necessary because there is no indication of type in the protologue. Of the five species described for the genus *Pachyglossum*, *Oxypetalum pachyglossum* Decne. is selected, and of the 10 species described for the genus *Schizostemma*, *Schizostemma parviflora* Decne. (= *Oxypetalum parviflorum* (Decne.) Decne.) is selected.

Key for the Species of *Oxypetalum* in Rio de Janeiro, Brazil

1a. Corpusculum with membranaceous appendage at apex . . . . . 2. *O. appendiculatum*
1b. Corpusculum without membranaceous appendage at apex.
  2a. Style-head usually crateriform or coroniform at apex or from the middle, usually elongate and exserted, more rarely laciniate or bifid, in this case with branches laminar or planar.
    3a. Corpusculum slightly longer than pollinia; caudicles with included teeth . . . . . 6. *O. costae*
    3b. Corpusculum conspicuously longer than pollinia; caudicles with free teeth.
      4a. Corona lobes trullate or ± trullate; corpusculum ± claviform . . . . . 18. *O. regnellii*
      4b. Corona lobes ± rectangular, rectangular or oblong; corpusculum linear, rectangular-elongate, oblong and laminar.
        5a. Leaf blades pubescent, tomentose, woolly or tawny-tomentose.
          6a. Style-head usually crateriform or coroniform at apex or up the middle . . . . . 9a. *O. insigne* var. *insigne*
          6b. Style-head, bifid to middle, 2 branchlets divaricate or lanceolate.
            7a. Leaves adaxially pubescent, abaxially tomentose; caudicles appressed at base of the corpusculum; pollinia 3 to 4× shorter than corpusculum . . . . . 19. *O. schottii*
            7b. Leaves tawny-tomentose to woolly; caudicles not appressed at base of the corpusculum; pollinia to 2× shorter than corpusculum or equal or approximately equal in size to corpusculum.
              8a. Leaf blades abaxially whitish lanose; corolla lobes reflexed, with smooth trichomes; corona lobes 1.8–2 × 1.3–1.8 mm, adaxially with tooth reaching up to middle of the corona lobe; corpusculum 0.90–0.99 × 0.26–0.30 mm, ossiform, thickened in lateral view, not laminar, curved; pollinia equal or approximately equal in size of corpusculum . . . . . 15. *O. pannosum*
              8b. Leaf blades abaxially fulvous-woolly; corolla lobes spreading, with verrucose trichomes; corona lobes 3–4.5 × 1.5–2 mm, tooth adaxially present, longer than corona lobe; corpusculum 1.10–1.20 × 0.30–0.35 mm, linear or oblong, thin, laminar, planar; pollinia 2× shorter than corpusculum . . . . . 11. *O. lanatum*
        5b. Leaf blades glabrous, glabrescent, or puberulent.
          9a. Corolla lobes adaxially glabrous; corpusculum 1.53–1.83 mm; style-head usually with planar apex shell-shaped or bifid with branches divaricate or erect . . . . . 9b. *O. insigne* var. *glabrum*

9b. Corolla lobes adaxially sparsely pubescent; corpusculum 1.23–1.35 mm; style-head usually planar with 2 branches trilobate at apex . . . . . 9c. *O. insigne* var. *glaziovii*

2b. Style-head usually elongate, exserted, in this case bifid and with branches linear or filiform, thick or not, rarely included or shortly exserted, ± entire, emarginate.

10a. Style-head included or shortly exserted.

11a. Leaves glabrous; corolla lobes spreading; peduncle 16–53(81) mm; corpusculum erect in side view, thick, not laminar; caudicles descending, with teeth included . . . . . 14. *O. pachyglossum*

11b. Leaves adaxially lanose or pubescent, abaxially lanose; corolla lobes reflexed; peduncle 0.4–1.5 mm, corpusculum curved in side view, not thick, laminar; caudicles horizontal, with teeth curved and free . . . . . 20. *O. sublanatum*

10b. Style-head usually elongate and exserted.

12a. Caudicles with teeth included.

13a. Corona lobes adaxially without tooth; anther wings shorter than the dorsum; caudicles descending or oblique; style-head bifid or ± entire, usually twisted . . . . . 1a. *O. alpinum* var. *alpinum*

13b. Corona lobes adaxially with tooth; anther wings longer than the dorsum; caudicles horizontal or ± horizontal; style-head bifid or bilobate and not twisted.

14a. Decumbent shrub; corona adaxially with an inconspicuous tooth; caudicles broad and planar; style-head bifid from the middle . . . . . 16. *O. patulum*

14b. Twining vine; corona adaxially with a conspicuous tooth; caudicles filiform; style-head bifid or bilobate at apex . . . . . 8. *O. glaziovii*

12b. Caudicles with teeth free and usually curved.

15a. Pollinia sigmoid, thin at tip.

16a. Corona enclosing only partially the connective appendage of the anthers; corpusculum curved in lateral view, abaxially with the split broad at apex; found along the Brazilian coast in restingas, disturbed forest, and secondary rainforest, from sea level to 1800 m elevation . . . . . 4a. *O. banksii* subsp. *banksii*

16b. Corona enclosing the connective appendage of the anthers; corpusculum erect or suberect in lateral view, abaxially with the split narrow at apex; found only in restingas from Rio de Janeiro, usually at sea level . . . . . 4b. *O. banksii* subsp. *corymbiferum*

15b. Pollinia cylindrical, oblong or ± elliptical, rounded at tip.

17a. Corpusculum laminar, usually thin.

18a. Corolla lobes adaxially verrucose or papillose-verrucose . . . 13. *O. molle*

18b. Corolla lobes adaxially puberulent, rarely ± glabrous.

19a. Corolla lobes linear-oblong; corona lobes rotund or ± rhomboidal; corpusculum 3× longer than the pollinia . . . . . 7. *O. glaziovianum*

19b. Corolla lobes linear-lanceolate or ± linear; corona lobes flabellate; corpusculum less than 2× longer than pollinia.

20a. Calyx lobes with 1 colleter below each sinus within; corona lobes adaxially always lacking tooth; caudicles long-stipitate . . . . . 5a. *O. cordifolium* subsp. *brasiliense*

20b. Calyx lobes with fascicle of colleters below each sinus within; corona lobes adaxially with tooth; caudicles short-stipitate . . . . . 5b. *O. cordifolium* subsp. *pedicellatum*

17b. Corpusculum linear or ± oblong, usually thick.

21a. Corolla lobes very elongate, 25–34 mm . . . . . 3. *O. arachnoideum*

21b. Corolla lobes short, 4.5–15 mm.

22a. Corolla rotate; corona lobes ± rhombic, adaxially tooth absent . . . . . 17. *O. pilosum*

22b. Corolla campanulate or ± campanulate; corona lobes oblong, ± rectangular, ± oblong, trilobate, adaxially with tooth present or process thickened, rounded or heart-shaped.

23a. Corona lobes oblong, ± oblong, ± rectangular or rectangular, adaxial tooth linear or filiform; style-head very elongate, 9–11 mm.
24a. Corolla tube glabrous at throat; corona with adaxial tooth surpassing tip of corona lobe; corpusculum as long as pollinia . . . . . 12. *O. lutescens*
24b. Corolla tube barbellate at throat; corona with adaxial tooth not reaching tip of corona lobe; corpusculum longer than pollinia . . . . . 10. *O. jacobinae*
23b. Corona lobes trilobate with adaxial process thickened, heart-shaped or rounded; style-head shortly elongate, 4–6 mm . . . . . 21. *O. wightianum*

1. Oxypetalum alpinum (Vell.) Fontella & E. A. Schwarz, Bol. Mus. Bot. Munic. 61: 2. 1984. *Asclepias alpina* Vell., Fl. Flumin. [Text]: 116. 1829. TYPE: Brazil. plate 57, J. M. C. Vellozo, Fl. Flumin. Icones 3, 57. 1831 (lectotype, designated by Fontella-Pereira & Schwarz, 1984a: 2, pl. 57).

1a. Oxypetalum alpinum (Vell.) Fontella & E. A. Schwarz var. **alpinum.** *Asclepias nitida* Vell., Fl. Flumin. [Text]: 116. 1829. TYPE: Brazil. pl. 59, J. M. C. Vellozo, Fl. Flumin. Icones 3: 59. 1831 (lectotype, designated by Fontella-Pereira & Schwarz, 1984a: 2, pl. 59).

Oxypetalum umbellatum Gardner in Hook., London J. Bot. 1: 179. 1842. *Gothofreda umbellata* (Gardner) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. "dry bushy places at Rio Comprido, near Rio de Janeiro," s.d., *G. Gardner 77* (holotype, K!).

Oxypetalum umbellatum var. *puberulum* Hoehne, Commiss. Linhas Telegr. Estratég. Matto Grosso Amazonas 38(1): 20. 1916. TYPE: Brazil. *Herbário RB 2490* (holotype, RB!).

Twining vine, branches glabrescent. Leaves with petiole 1–2 cm, pubescent, with 4 colleters at blade base; blades 5.2–6.5 × 1.3–2.1 cm, narrowly oblong to lanceolate, brochidodromous, basally cordate, apically acuminate, adaxially glabrous or glabrescent, abaxially puberulent. Inflorescence 12- to 14-flowered, sciadioidal; peduncle (0.4)1–2.7 cm, pubescent; pedicels 5–10 mm, pubescent. Floral buds 0.6–0.8 cm, conical; calyx lobes 1.5–3 × 0.4–1 mm, with 1 colleter below each sinus within, lanceolate, glabrous outside, pubescent within, with smooth trichomes; corolla tubular to narrowly campanulate, tube 2–2.5 mm, pubescent outside, glabrous inside, lobes 4.5–6.5 × 0.6–2 mm, linear-lanceolate to lanceolate, spreading and twisted at apex, abaxially pubescent, adaxially barbellate at base and papillose on the central, apical, and lateral parts, with smooth trichomes (68)96–138(276) μm; corona lobes 3.5–4.5 × 1.2–1.5 mm, linear-oblong to rectangular, usually exserted and obscuring the anthers, with emarginate or truncate apex, tooth adaxially absent; gynostegium 1–1.5 mm high, ± sessile; anthers ± triangular, with wings 0.6–0.9 mm, shorter than dorsum; connective appendage 0.5–0.6 mm, ovate; corpusculum 0.3–0.4 × 0.1–0.12 mm, oblong or elliptical, thin, not laminar and planar; caudicles 0.1–0.2 mm, descending or oblique, fixed on base of corpusculum, shortly stipitate, teeth indistinct and included; pollinia 0.44–0.6 × 0.16–0.18 mm, oblong to ± elliptical, slightly longer than corpusculum; style-head 3.5–8 mm, cylindrical, elongate and exserted, bifid or ± entire, usually twisted. Follicle 7–8.2 × 0.9–1.2 cm, fusiform, with acuminate tip, glabrescent to tomentose; seeds 6–7.5 × 2.8–3 mm, ovate, slightly verrucose, coma 2.5–3 mm.

Phenology. Collected in flower from January to December; in fruit in February, April, June, and December.

Distribution. Brazil: Espírito Santo, Minas Gerais, and Rio de Janeiro.

Oxypetalum alpinum is usually found in seasonal wetlands from restinga, restinga forest, and secondary Atlantic rainforest from sea level to 790 m in Brazil. *Oxypetalum alpinum* var. *alpinum* is quite similar to *O. pachyglossum* in lamina shape and overall appearance. The taxa are distinguished by the campanulate corolla with the tube adaxially glabrous of *O. alpinum* var. *alpinum* (vs. the corolla with the tube adaxially barbellate of *O. pachyglossum*) and by the style-head longer and exserted of the former (vs. the style-head shorter and usually included of the latter).

Specimens examined. BRAZIL. **Espírito Santo:** Linhares, Res. Flor. Cia. Vale do Rio Doce, *Martinelli et al. 1861* (RB). **Minas Gerais:** Marliéria, Parq. Est. Rio Doce, Lagoa Carioca, *Lombardi 1289* (BHCB). **Rio de Janeiro:** Angra dos Reis, Ilha Grande Res. Biol. Est. Praia do Sul, *Araujo et al. 6699* (GUA); Angra dos Reis, *Brade 20515* (RB); Barra do Piraí, Ipiabas, *Laclette s.n.* (R-37324); Campos dos Goytacazes, Lagoa da Saudade, *Araujo 4557 & Maciel* (GUA); Campos dos Goytacazes, Lagoa das Pedras,

Pedrosa 841 & Casari 942 (GUA); Carapebus, Carapebus, *Konno 607* (SP); Praia de Carapebus, *Lima 676* (RB); Itatiaia, *Markgraf & Brade 21252* (RB); Magé, Cachoeiras de Macacú, Res. Ecol. Est. Paraíso, Faz. Curtume Carioca, Pedra Ferro, *Vaz 959* (RB); Magé, Polder de Pilar, *Lutz 1541* (R); Maricá, *Souza et al. 1990* (R); Amil, *Pereira 648* (HB, RB); Natividade, Caiçaras, *Duarte 1116* (RB); Passa Tres, *Mello s.n.* (RB-40556, RFA); Petrópolis, Mata do Judeo, Petrópolis, *Sucre 4241 & Braga 1198* (RB); Rio de Janeiro, *Saint-Hilaire catal. B2. n.14* (F); Baixada de Jacarepaguá, Lagoa de Marapendi, *Araujo 5427* (GUA, NY); Santa Maria Madalena, Estação de Desengano, Faz. Sta. Monica, *Occhioni 1177* (RB); Silva Jardim, Res. Biol. Poço das Antas, *Araujo 4011* (GUA); Silva Jardim, *Lima et al. 4580* (RB); Teresópolis, Faz. Comary, *Occhioni 1282* (F, RB). State not known, *Schott 5385* (B, NY).

2. Oxypetalum appendiculatum Mart., Nov. Gen. Sp. Pl. 1: 48, t. 30. 1824. *Gothofreda appendiculata* (Mart.) Kuntze, Revis. Gen. Pl. 2: 419. 1891. TYPE: Brazil. Minas Gerais: "habitat inter frutices prope Lagoa Dourada inter S. João d El Rey et Villam Ricam alibique in montosis," s.d., *C. F. P. von Martius s.n.* (holotype, M!).

Oxypetalum siliculae E. Fourn., Fl. Bras. 6(4): 266. 1885. *Gothofreda siliculae* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. São Paulo: "ad S. Carlos, Januario," *L. Riedel 2189*, p.p. (holotype, LE!).

Twining vine, branches tomentose to pubescent. Leaves with petiole 1–4 cm, tomentose, with 4 colleters at blade base; blades (3)5–10.8(13) × 2.2–4.5(5) cm, oblong to ovate-lanceolate, camptobrochidodromous, basally cordate, apically acuminate, adaxially tomentose to pubescent, abaxially tomentose. Inflorescence 1- to 8-flowered, sciadioidal; peduncle 4–21 mm, tomentose; pedicels 3.6–14 mm, tomentose to pubescent, 1- or 3-bracteolate. Floral buds 1.3–1.7 cm, ovate; calyx lobes 4.5–11 × 0.5–0.7 mm, with 2 or 3 colleters below each sinus within, linear to linear-lanceolate, pubescent outside, glabrous inside, with verrucose trichomes 71–112 μm; corolla campanulate, tube 4–7 mm, pubescent outside, puberulent inside, lobes 11–24 × 2.5–4 mm, triangular-elongate, usually erect and abaxially pubescent to tomentose, adaxially glabrous and slightly verrucose at throat, with verrucose trichomes 33–34 μm; corona lobes 2.5–3 × 2–2.2 mm, ovate-rectangular to oblong, obscuring the anthers, tooth adaxially absent; gynostegium 2.5–3 mm high, inside the corolla tube, sessile; anthers trapezoidal, with wings 0.5–1 mm, longer than dorsum; connective appendage 1.5–1.7 mm, cordiform to triangular; corpusculum 0.84–0.92 × 0.10–0.12 mm, in frontal view, with a membranaceous appendage rounded at apex, thick, not laminar, planar, ± linear; caudicles 0.24–0.34 mm, horizontal, with teeth lateral, curved and free, shortly stipitate; pollinia 0.56–0.66 × 0.14–0.16 mm, oblong, equal or slightly longer than corpusculum; style-head 10–11 mm, bifurcate in filiform, erect branches. Follicle 6.1–9.8 × 0.9–1.3 cm, ovate to fusiform, tomentose; seeds 5–5.9 × 3–4 mm, ovate, coma 2–2.5 cm.

Phenology. Collected in flower the entire year; in fruit from January to June and August to November.

Distribution. Brazil: Mato Grosso, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo. Also in Argentina, Bolívia, Paraguay, and Uruguay (Fontella-Pereira et al., 2004).

Oxypetalum appendiculatum is found in rainforest, secondary rainforest, gallery forest, and high-altitude grasslands in Rio de Janeiro. In other Brazilian states, it occurs in rainforest, rainforest with *Araucaria*, open secondary vegetation, seasonal forest, disturbed seasonal forest, cerradão, cerrado-cerradão, cerrado, campo rupestre, and grasslands campo from 200 to 1821 m. It is the most distinctive *Oxypetalum* species from Brazil and has the gynostegium inside the corolla tube, the lobes erect, and the corpusculum with a membranaceous appendage rounded at its apex.

Specimens examined. BRAZIL. **Minas Gerais:** Ouro Preto, Camarinhas, *Marquete et al. 293* (R, RB). **Paraná:** Guarapuava, Lagoa Sêca, *Hatschbach 19783 & Guimarães* (MBM, RFA, S). **Rio de Janeiro:** Itatiaia, Parq. Nac. Itatiaia, *Occhioni 1223* (RB); Itatiaia, Monteserrat, *Porto 1023* (RB); Nova Friburgo, Pico Nova Caledônia, *Lima et al. 2529* (INPA, NY, RB); Petrópolis, Araras, Vale da Cachoeira, *Ferreira 1085* (RB); Petrópolis, Serra das Araras, *Occhioni 1493* (RFA); Teresópolis, Condomínio Comary, Alfredo de Carvalho, *Marquete et al. 436* (R, RB); Teresópolis, Posse, Morro das Antenas de Televisão, *Sucre 2300 & Braga 143* (RB); **Rio Grande do Sul:** Porto Alegre, Chácara do Mostardeiro, Moinhos de Vento, *Pabst 5727* (HB). **Santa Catarina:** São Joaquim, Bom Jardim, Faz. Laranja, *Reitz & Klein 8190* (HBR). **São Paulo:** São José do Barreiro, Bocaina, *Rapini & Carvalho 931* (SPF). State not known: "Ex Herb. brasil. Regnelli" (S).

3. Oxypetalum arachnoideum E. Fourn., Fl. Bras. 6(4): 272. 1885. *Gothofreda arachnoidea* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 419. 1891. TYPE: Brazil. "ad Rio de Janeiro," s.d., *A. F. M. Glaziou 14091* (holotype, P!; isotypes, B!, R!, B photo at F!, HB!, RB!).

Oxypetalum grandiflorum E. Fourn., Fl. Bras. 6(4): 272. 1885. *Gothofreda grandiflora* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. São Paulo: "in capoeiras," s.d., *A. F. C. P. St.-Hilaire Cat. B1 n. 1507* (lectotype, designated by Fontella-Pereira, 1970: 2, P!, P photo at K!, RB!; isotype, P fragment at F!).

Twining vine, branches tomentose. Leaves with petiole 3.7–3.8 cm, pubescent to tomentose, with 3 or 4 colleters at blade base; blades 5.8–6.5 × 3.9–4.2 cm, brochidodromous, basally cordate, apically

acuminate or caudate, adaxially sparsely pubescent, abaxially pubescent. Inflorescence usually ca. 3-flowered, dichasial to sciadioidal-corymbiform; peduncle 3.5–5.4 cm, pubescent to tomentose; pedicels 19–25 mm, pubescent to tomentose, 2- or 3-bracteolate. Floral buds 1.8–3 cm, ovate-lanceolate; calyx lobes 4.2–4.8 × 0.8–1 mm, with 3 colleters below each sinus within, linear to lanceolate, pubescent to tomentose outside, glabrous inside, with smooth trichomes 105–148 μm; corolla tube 3–5 mm, shortly campanulate, puberulent to glabrate outside, glabrous inside, enclosing the anthers, lobes 25–34 × 2–2.4 mm, linear, very elongate, spreading, abaxially puberulent to ± glabrate, with smooth trichomes, adaxially glabrous; corona lobes 2.2–3.5 × 2.2–2.5 mm, oblong to rectangular, apex truncate or undulate, slightly longer than anthers, tooth adaxially absent; gynostegium 4–4.5 mm high, ± sessile; anthers ± trapezoidal, with wings 1–1.2 mm, longer than dorsum; connective appendage 1.2–1.5 mm, ovate; corpusculum 1.36–1.81 × 0.08–0.21 mm, linear in frontal view, thick, not laminar; caudicles 0.11–0.34 mm, horizontal, with teeth lateral, curved and free, shortly stipitate, inserted ± basally at corpusculum; pollinia 0.73–0.89 × 0.10–0.31 mm, oblong, shorter than corpusculum; style-head 7–7.5 mm, elongate and exserted, bifid from the middle, with filiform branches usually twisted. Fruit not seen.

Phenology. Collected in flower from March to April.

Distribution. Brazil: Bahia (Fontella-Pereira et al., 1989), Minas Gerais, Rio de Janeiro, and São Paulo.

Oxypetalum arachnoideum is found in secondary rainforest in Rio de Janeiro growing at 50 to 200 m and in gallery forest in Minas Gerais (Rapini et al., 2001). In Bahia it was collected in rocky thickets and along watercourses at ca. 1000 m. This species is readily recognized by its lobes that are rather elongate and usually twisted, corona with tooth adaxially absent, and style-head elongate with branches usually twisted. It is quite similar to *O. banksii* subsp. *banksii* in overall appearance. However, it differs from this taxon principally in its inflorescence being dichasial to sciadioidal-corymbiform (vs. cymoid to corymbiform), corolla lobes 25–34 mm (vs. 14–25 mm), and style-head bifid from the middle with twisted branches (vs. bifid from the middle with erect or divaricate branches).

Specimens examined. BRAZIL. **Bahia:** Serra Geral de Caitité, Caitité, Brejinhos das Ametistas, *Harley 21204* (K, RB). **Rio de Janeiro:** Niterói, Morro Cavalão, *Brade 10402* (F, R, S); Rio de Janeiro, Silvestre, Estrada Nova, *Duarte 4774 & Pereira* (F, HB, RB, RFA); Rio de Janeiro, Novo Mundo, *Kuhlmann 16349* (S, SP); Rio de Janeiro, Corcovado, *Ule s.n.* (R).

4. **Oxypetalum banksii** R. Br. ex Schult., Syst. Veg. ed. 15 bis [Roemer & Schultes] 6: 91. 1820. *Gothofreda banksii* (Schult.) Kuntze, Revis. Gen. Pl. 2: 419. 1891. TYPE: Brazil. Rio de Janeiro: *J. Banks & D. C. Solander 1768* (holotype, BM!, BM photo at RB!).

4a. **Oxypetalum banksii** subsp. **banksii** Schult. *Asclepias communis* Vell., Fl. Flumin. [Text]: 115. 1829. TYPE: pl. 53, J. M. C. Vellozo, 1831 (lectotype, designated by Rapini et al., 2001: 90, pl. 53 in Vellozo, 1831).

Oxypetalum maritimum Hook. & Arn., J. Bot. (Hooker) 1: 288. 1834. *Gothofreda maritima* (Hook. & Arn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Bahia: "in maritimis [as *Apocynum maritimum*] of *Salzmann's Herb. Brasil. 329*" (holotype, K!; isotype P!, P photo at RB!).

Twining vine, branches pubescent to tomentose. Leaves with petiole 0.6–6.3 cm, pubescent to tomentose, with ca. 4 colleters at blade base; blades 2.5–12.4 × 1.3–9.5 cm, ovate to oblong, rarely ± rotund, camptobrochidodromous, basally cordate or auriculate, apically acuminate or acute, adaxially glabrescent to sparsely pubescent, abaxially pubescent to tomentose. Inflorescence 1- to 12-flowered, cymoid to corymbiform; peduncle 0.3–5 cm, pubescent to tomentose; pedicels 10–20 mm, usually pubescent, 2- or 3-bracteolate. Floral buds 1.0–1.5 cm, conical; calyx lobes 3–4 × 1.0–1.5 mm, with 1 or 3 colleters below each sinus within, rarely colleters absent, linear-lanceolate or triangular-elongate, pubescent to tomentose outside, glabrescent inside, smooth trichomes 91–288 μm; corolla rotate to campanulate, tube 1.8–2.0 mm, puberulent outside, pubescent to tomentose within, lobes 14–25 × 2.0–3.0 mm, linear-lanceolate, reflexed and usually twisted at apex, abaxially pubescent to tomentose, adaxially puberulent to slightly papillose, with smooth trichomes (51)83–149 μm; corona lobes 2.8–3.0 × 2.2–2.5 mm, spathulate to oblong-spathulate, truncate to rounded and wrinkled at apex, exserted, sometimes reflexed, enclosing only partially the connective appendage of the anthers, tooth adaxially absent; gynostegium 2.3–2.5 mm high, sessile; anthers ± trapezoidal, with wings 1.6–2 mm, longer than dorsum; connective appendage 2–2.5 mm, ovate, exserted, higher than corona lobes; corpusculum 1.55–1.76 × 0.26–0.30 mm, ± claviform in frontal view with split broad at apex, curved in lateral view, thick, not laminar; caudicles 0.26–0.30 mm, horizontal, with teeth lateral, curved and free, shortly stipitate, inserted ± basally at corpusculum; pollinia 1.14–1.33 × 0.19–0.22 mm, sigmoid, thin at tip, usually shorter than corpusculum; style-head 0.5–1.5 mm, conical at base, elongate and exserted, bifid

from the middle, with filiform, erect or divaricate branchlets. Follicle 6.4–7.4 × 0.9–1.5 cm, fusiform, glabrous, with acuminate tip; seeds 5.5–6 × 2.8–3 mm, ovate, coma 2–2.5 cm.

Phenology. Collected in flower from January to December; in fruit from January to September.

Distribution. Brazil: Alagoas (Fontella-Pereira et al., 2004), Bahia, Espírito Santo, Minas Gerais, Paraná (Fontella-Pereira et al., 2004), Rio de Janeiro, Rio Grande do Sul (Fontella-Pereira et al., 2004), Santa Catarina, and São Paulo.

In Brazil, *Oxypetalum banksii* is found along the coast, usually in restingas, disturbed forest, and secondary rainforest from sea level to 1800 m. This species is readily recognized by its corolla lobes reflexed and usually twisted at the apex, corona lobes not enclosing the gynostegium and exserted, tooth adaxially absent, and sigmoid pollinia. The subspecies is quite similar to *O. arachnoideum* in overall appearance. However, it differs from this species principally in its inflorescences being cymoid to corymbiform (vs. dichasial to sciadioidal-corymbiform), corolla lobes 14–25 mm (vs. 25–34 mm), and style-head bifid from the middle with erect or divaricate branches (vs. bifid from the middle with usually twisted branches).

Specimens examined. BRAZIL. **Bahia:** Vale do Rio Mucuri, BR 101, *Belém 3885* (NY). **Espirito Santo:** Guarapari, *Occhioni 7219* (RFA). **Minas Gerais:** Caratinga, Est. Biol. Caratinga, Faz. Montes Claros, Rafael, *Lombardi 3675* (BHCB). **Rio de Janeiro:** Angra dos Reis, Ilha Grande, Res. Biol. Est. Praia do Sul, *Araujo et al. 5699* (GUA); Araruama, near Lagoa Vermelha, *Araujo 2127 & Maciel* (GUA); Arraial do Cabo, Res. Ecol. Est. Massambaba, near Lagoa Salgada, *Araujo 8713 & Maciel* (GUA); Barra do Piraí, Ipiabas and Faz. Prosperidade, *Carauta 5276 & Peixoto 492* (GUA); Búzios, Armação dos Búzios, restinga da Faz. Junqueira, Rasa, *Martinelli et al. 1167* (RB); Cabo Frio, Rio Una, *Araujo 5632* (GUA); Campos dos Goytacazes, *Vianna et al. 1383* (GUA); Casimiro de Abreu, betw. Barra de São João–Rio das Ostras, *Martinelli et al. 5669* (NY); Cordeiro, Santa Maria Madalena, em direção à estrada para Bonsucesso, *Klein et al. 742* (RB); Duque de Caxias, Res. Petrobras, Largo do Sangradouro, *Silva Neto et al. 14000* (RB); Itatiaia, Parq. Nac. Itatiaia, *Braga 2899* (RB); Macaé, Frade to Usina Elétrica, *Marquete 407* (RB); Magé, Piabetá, Km 135 da Rodovia Rio–Teresópolis, Parq. Estrela, Portinho, *Giordano 1685* (RB); Maricá, Barra de Maricá, *Lewinson & Monteiro 18122* (UB); Mendes, *Vidal s.n.* (R); Resende-Formoso, Resende, *Pirani 2907 & Mello Silva* (NY); Niteroi, Itaipu, *Santos 2456, Fromm Trinta 2349, Sacco 2551* (R); Parati, Sono, *Marquete, R. 416* (HRB, RB); Paracambi, *Carauta et al. 6380* (GUA); Petrópolis, Araras, Vale das Videiras, Miguel Pereira, *Marquete et al. 286* (R, RB); Quissamã, Visqueiro, *Marquete et al. 403* (R, RB); Rio Bonito, *Occhioni 3858* (RFA); Rio das Ostras, Praia do Norte, *Marquete et al. 323* (R, RB); Rio de Janeiro, Praia de Sernambetiba, Recreio dos Bandeirantes, *Smith et al. 6340* (R); Rio de Janeiro, Pão de Açucar, *Carauta et al. 3420* (GUA); São João da Barra, Silva Jardim-Araruama, *Fromm 1321, Santos 1343, Trinta 247* (R); Saquarema, Sambaqui da Beirada, *Araujo 8078* (GUA); Silva Jardim, Res. Biol. Poços das Antas, Faz. Portuense, *Martinelli 8463 & Lima* (RB). **Santa Catarina:** Itajaí, Cabeçudos, *Occhioni 3491* (RFA). **São Paulo:** Ubatuba, Rio Acaraú, 8 km S of Inst. Oceonográfico, *Fontella 77 & Moura* (HRCB, SP).

4b. Oxypetalum banksii subsp. **corymbiferum** (E. Fourn.) Fontella & C. Valente, Anais Acad. Brasil. Ci. 43(1): 177. 1971. *Oxypetalum corymbiferum* E. Fourn., Fl. Bras. 6(4): 267. 1885. *Gothofreda corymbifera* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 419. 1891. TYPE: Brazil. Rio de Janeiro, *A. F. C. P. St.-Hilaire 212* (holotype P!, P photo at RB!).

Oxypetalum schenckii K. Schum., Bot. Jahrb. Syst. 25(60): 20. 1898. TYPE: Brazil. "in Brasiliae civitate Rio de Janeiro prope Cabo Frio in formatione Restinga dicta scandens," *H. Schenck 3809* (lectotype, designated by Fontella et al., 1971: 12, B not seen, B photo at RB!).

Oxypetalum marambaiense Occhioni, Leandra 1(1): 7, pl. 1. 1971. TYPE: Brazil. "in Brasiliae in civitate Rio de Janeiro, formatione Restinga dicta Marambaia," 20 Jan. 1970, *P. Occhioni 4184* (holotype, RFA 9550!).

Twining vine, branches tomentose to pubescent. Leaves with petiole 0.5–3.8 cm, pubescent to tomentose, with 4 colleters at blade base; blades 2.5–6.8 × 1–5 cm, ovate, ovate-oblong, camptobrochidodromous, apically acuminate or mucronate, basally cordate, abaxially pubescent, adaxially tomentose to pubescent. Inflorescence 4- to 19-flowered, cymoid to corymbiform; peduncle 2–23 cm, tomentose; pedicels 10–16 mm, tomentose or pubescent, 2- or 3-bracteolate. Floral buds 0.6–0.9 cm, ovate with apical elongations; calyx lobes 2–2.5 × 0.7–0.9 mm, with 1 colleter below each sinus within, linear-lanceolate or triangular-lanceolate, abaxially pubescent, adaxially glabrous at base to slightly puberulent toward apex, with verrucose trichomes 79–201 µm; corolla rotate to campanulate, tube 1.5–1.7 mm, pubescent or tomentose outside, papillose to puberulent within, with 5 concavities inside at base, lobes 8–16 × 2.5–3 mm, linear-lanceolate, acute or obtuse at apex, reflexed, spiralled, abaxially pubescent, adaxially puberulent or papillose, with verrucose trichomes (41)67–118 µm; corona lobes 2.5–4.5 × 1.5–2 mm, oblong to ± spathulate, ovate-oblong or ± ovate, truncate to rounded at apex, exserted, forming a fleshy and inconspicuous protuberance at junction of basal corona and stamimal column, tooth adaxially absent enclosing the connective appendage of the anthers; gynostegium 2.2–2.6 mm high, sessile; anthers ± rectangular, with wings 2.3–2.7 mm, usually equal to the dorsum; connective appendage 1.3–1.5 mm, ovate, usually enclosed by the corona; corpusculum 1.06–1.65 × 0.12–0.22 mm, in frontal view linear to

± linear, with narrow split at apex, ± erect in lateral view, thick, not laminar, curved; caudicles 0.26–0.34 mm, horizontal, shortly pedicellate, inserted ± basally at corpusculum, with teeth lateral, curved and free, pollinia 0.94–1.17 × 0.12–0.22 mm, sigmoid, thin at tip, usually shorter than corpusculum; style-head 1.8–2 mm, conical at base, elongate and exserted, usually bifid from the middle, with filiform, erect or divaricate branchlets. Follicle 6.3–8 × 1.1–1.5 cm, fusiform or ovate, with acuminate tip, glabrous or puberulent; seeds 4.5–6 × 1.3–2.5 mm, ovate, subovate, coma 3–3.5 mm.

Phenology. Collected in flower from January to December; in fruit in May and from August to November.

Distribution. Brazil: Rio de Janeiro.

Oxypetalum banksii subsp. *corymbiferum* is endemic to Rio de Janeiro, growing in open restinga, usually at sea level. It is quite similar to the typical subspecies in its lamina shape, reflexed corolla lobes, and sigmoid pollinia. These taxa are usually distinguished by the corona including entirely or partially the membranaceous appendages of the anthers (vs. corona not included and the membranaceous appendage exserted in subsp. *banksii*).

Specimens examined. BRAZIL. **Rio de Janeiro:** Araruama, Zacara, Brejo do Espinho, *Araujo et al. 5125* (GUA); Arraial do Cabo, Figueiras, Arraial do Cabo, *Marquete et al. 320* (R, RB); Cabo Frio, Restinga de Massambaba, Figueiras, *Marquete et al. 139* (HRB, RB); Cabo Frio–Arraial do Cabo, Iguaba, Cabo Frio, *Fontella 2263* (NY, RB); Campos dos Goytacazes, Praia de São Tomé, *Araujo 7100 & Maciel* (GUA); Maricá, barra de Maricá, *Landrum 4171* (NY); Rio das Ostras, Mar do Norte, Km 106, Rodovia RJ, *Marquete et al. 385* (RB); Rio de Janeiro, Baixada de Jacarépagua, Parq. Mansões, *Araujo 7836 & Pereira* (GUA); Recreio dos Bandeirantes, 3 km N of Sernanbetiba, *Fontella et al. 409* (RB); São João da Barra, Gruçaí, *Rocha et al. 869* (GUA); Saquarema, Res. Ecol. Est. Jacarepiá, *Konno 470* (SP).

5. Oxypetalum cordifolium (Vent.) Schltr., Symb. Antil. 1(2): 269. 1899. *Gothofreda cordifolia* Vent., Choix Pl. 36: t. 60. 1808. TYPE: Paris. tab 60 in Ventenat, Choix Pl., 1899 (lectotype, designated by Fontella-Pereira et al., 2005: 104).

5a. Oxypetalum cordifolium subsp. **brasiliense** (Decne.) Fontella & Goyder, Kew Bull. 60(1): 108. 2005. *Oxypetalum riparium* var. *brasiliense* Decne., Prodr. (DC.) 8: 583. 1844. TYPE: Brazil. Minas Gerais: "in Brasiliae sylvis caldasis inter virgulta, prope praesidium S. João-Baptista," *C. F. P. Martius 1078* (holotype, M!, M photo at K!). Figure 1.

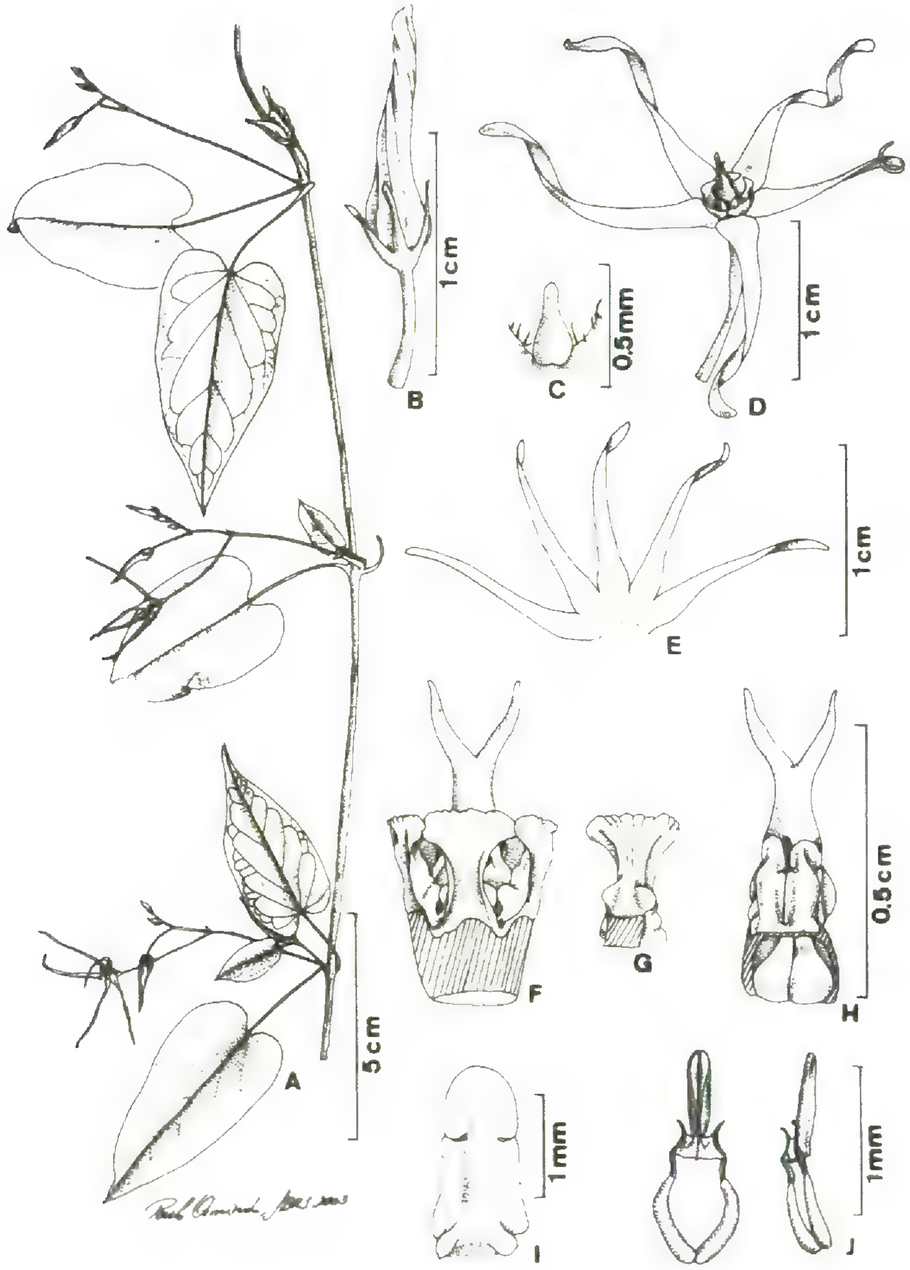
Oxypetalum subriparium Malme, Ark. Bot. 25A(7): 14, fig. 8. 1932. TYPE: Brazil. Minas Gerais: Viçosa, 28 Mar. 1839, *Ynes Mexia 4527* (holotype S!; isotype K! [specimen on righthand sheet]).

Twining vine, branches puberulent to glabrescent. Leaves with petiole 1–2 cm, puberulent with ca. 2 or 4 colleters at blade base; blades 4–5.5 × 1.5–2.5 cm, ovate-triangular, ovate, or cordiform, camptobrochidodromous, apically acute or acuminate, basally cordate or auriculate, adaxially puberulent, abaxially pubescent or puberulent. Inflorescence 2- to 3-flowered, cymoid to corymbiform; peduncle 12–20 mm, puberulent; pedicels 14–17 mm, puberulent. Floral buds 0.8–1 cm, lanceolate or ovate-lanceolate; calyx lobes 3–4 × 0.5–0.6 mm, with 1 colleter below each sinus within, linear-lanceolate, abaxially puberulent, adaxially ± glabrous, verrucose trichomes 102–162 µm; corolla campanulate, tube 3–4 mm, puberulent outside, glabrous inside, lobes 10–12 × 0.8–2 mm, linear-lanceolate or ± filiform, spreading, twisted, abaxially pubescent or puberulent, adaxially ± glabrous to papillose, verrucose trichomes 59–105 µm; corona lobes 1.5–2 × 0.4–0.5 mm, flabelliform, apex dilated and slightly crenate, with 2 lateral fleshy appendages at base adaxially always lacking the tooth; gynostegium 1.8–2 mm high, ± sessile; anthers rectangular, with wings 1.4–1.5 mm, equal in size to the dorsum; connective appendage 0.5–1 mm, ovate to ± rotund; corpusculum 0.71–0.80 × 0.12–0.15 mm, ± linear in lateral view, longer than pollinia but not 2× longer, thin, laminar, planar; caudicles 0.25–0.30 mm, horizontal, fixed on base of corpusculum, broad, long-stipitate, teeth lateral, curved, free; pollinia 0.59–0.75 × 0.10–0.15 mm, cylindrical-oblong, clearly curved, equal in size; style-head 4–5 mm, elongate and exserted, ± thickened, bifid from the middle, with filiform branches. Follicle 13–14 × 1.3–1.5 cm, fusiform or oblong-ovate with elongate-acuminate tip, puberulent; seeds 8–10 × 3–5 mm, spathulate-curved.

Phenology. Collected in flower from February to June and October to November; in fruit from April to May, August, and December.

Distribution. Brazil: Minas Gerais and Rio de Janeiro.

Oxypetalum cordifolium occurs in southeastern Brazil in Rio de Janeiro and Minas Gerais, usually in low montane rainforest and disturbed forest, from 300–800 m. *Oxypetalum cordifolium* subsp. *brasiliense* is quite similar to *O. cordifolium* subsp. *pedicellatum* in lamina shape and overall appearance. The taxa are distinguished usually by the calyx lobe sinuses with no colleter or one colleter (vs. eight or nine colleters), corona adaxially without tooth (vs. adaxially with tooth), and pollinia long-stipitate and clearly curved in *O. cordifolium* subsp. *brasiliense* (vs.



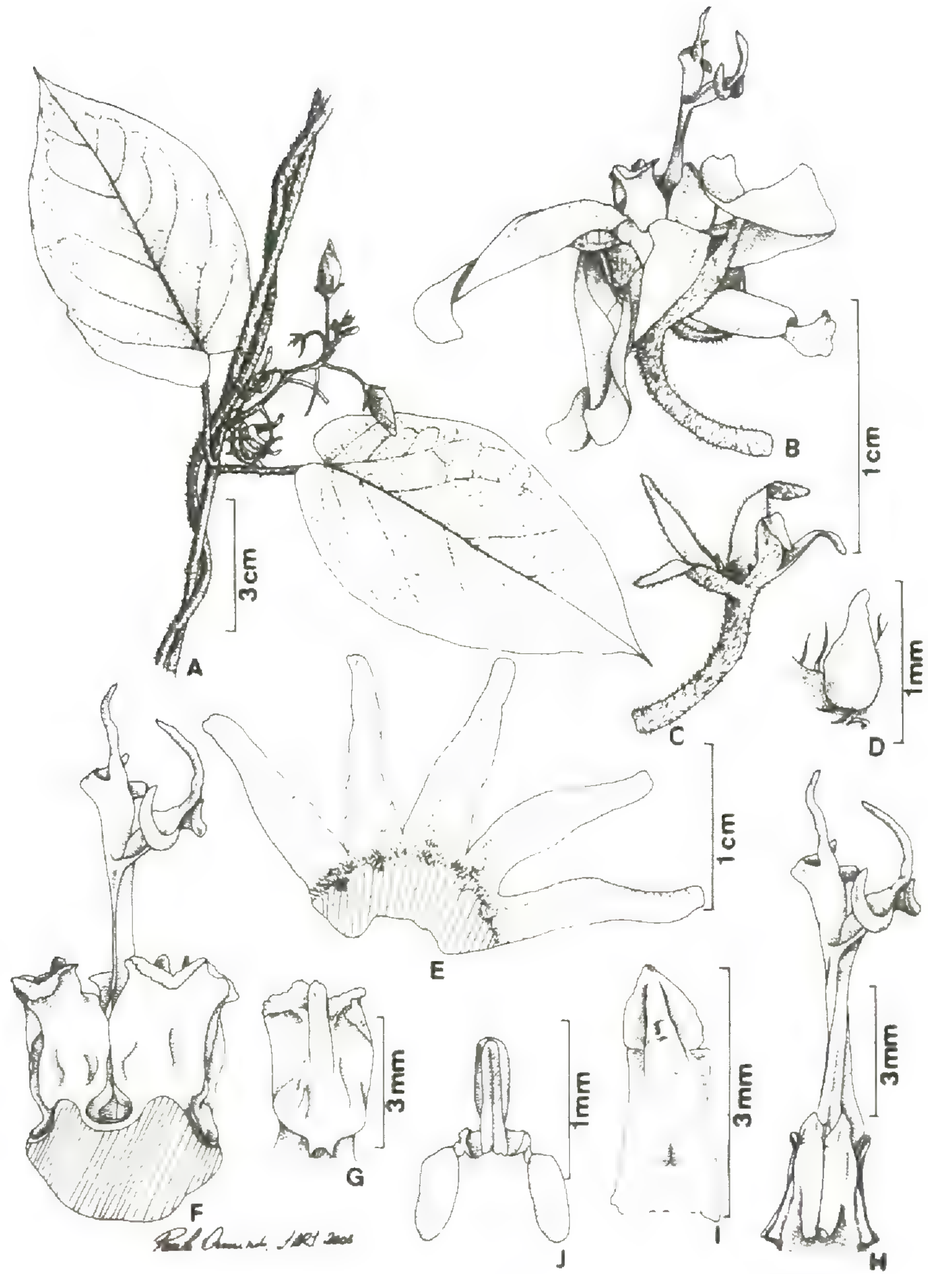


Figure 1 (left). *Oxypetalum cordifolium* subsp. *brasiliense*. —A. Flowering branch. —B. Floral bud. —C. Colleter in calyx lobe sinus. —D. Flower. —E. Corolla lobes, adaxial view. —F. Corona and stylar appendage with corolla lobes removed. —G. Corona lobe, adaxial view. —H. Gynostegium. —I. Anther, abaxial view. —J. Pollinarium, frontal view and lateral view. Drawn from *N. Marquete et al. 447* by P. Ormindo.

Figure 2 (right). *Oxypetalum costae*. —A. Flowering branch. —B. Flower. —C. Calyx. —D. Colleter in calyx lobe sinus. —E. Corolla lobes, adaxial view. —F. Corona and stylar appendage with corolla lobes removed. —G. Corona lobe, adaxial view. —H. Gynostegium. —I. Anther, abaxial view. —J. Pollinarium. Drawn from *Braga et al. 1570* by P. Ormindo.

pollinia shortly stipitate and slightly curved in subsp. *pedicellatum*).

Specimens examined. BRAZIL. **Minas Gerais:** Marliéria, Parq. Est. Rio Doce, *Lombardi 2866* (BHCB). **Rio de Janeiro:** Barra do Piraí, *Gehrt & Hoehne 17324* (S, SP); Petrópolis, Caiçaras, *Duarte 1117* (RB); Resende, Serrinha, *Marquete et al. 447* (R, RB); Rio de Janeiro, Camorim, *Occhioni & Pereira 5712* (HB, RB).

5b. Oxypetalum cordifolium (Vent.) Schltr. subsp. **pedicellatum** (Decne.) Fontella & Goyder, Kew Bull. 60(1): 108. 2005. *Oxypetalum pedicellatum* Decne., Prodr. (DC.) 8: 582. 1844. *Gothofreda pedicellata* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. "in Brasilia," s.d., *A. F. M. Glaziou 8795* (holotype G!, G photo at RB!; isotypes, BM!, R!, S!).

Oxypetalum pedicellatum Decne. var. *itatiaiense* Occhioni, Dusenia 3(3): 201. 1952. TYPE: Brazil. Rio de Janeiro: Itatiaia, Km 4–5, 28 May 1949, *P. Occhioni 1227* (holotype, RB 66461!).

Twining vine, branches glabrescent. Leaves with petiole 2–7.2 cm, puberulent, with 4 or 7 colleters at blade base; blades 6–9(12.5) × 3–5(8) cm, ovate to lanceolate, camptobrochidodromous, apically acuminate or elongate-acuminate, basally cordate to auriculate, usually with conspicuous reticulate nerving, puberulent adaxially and abaxially. Inflorescence 2- to 8-flowered, cymoid to corymbiform; peduncle 2–5(20) mm, puberulent; pedicels 17–23 mm, puberulent. Floral buds 0.6–1 cm, triangular or linear; calyx lobes 2–2.7 × 0.5–0.8 mm, with fascicle of 6 or 9 colleters below each sinus within, lanceolate, abaxially pubescent, adaxially puberulent, with verrucose trichomes 98–119 μm; corolla campanulate, tube 2–2.7 mm, puberulent to pubescent outside, glabrescent to puberulent inside, lobes 8–14 × 1.2–2 mm, ± linear, spreading, abaxially pubescent, adaxially puberulent, with verrucose trichomes 54–71 μm; corona lobes 1–2.5 × 0.7–1 mm, flabellate, apex truncate to retuse, thickened, verrucose, tooth adaxially present, shorter than corona lobe; gynostegium 1–2 mm high, sessile; anthers rectangular, with wings 0.7–1 mm, longer than dorsum; connective appendage 0.6–0.8 mm, oblong, ovate or elliptic; corpusculum 0.84–0.92 × 0.16–0.18 mm, linear-oblong, thin, laminar, planar, longer than pollinia but not 2× longer; caudicles 0.04–0.06 mm, fixed on base of corpusculum, horizontal, short-stipitate, teeth lateral, curved, free; pollinia 0.50–0.58 × 0.08–0.14 mm, cylindrical, slightly curved, sessile; style-head 2.6–4.5 mm, cylindrical, elongate and exserted, bifid from the middle, divergent. Follicle 6.5–13.8 × 0.9–1.2 cm, oblong-lanceolate, acuminate to apex, narrow and rounded at base, minutely pubescent; seeds 0.7–1.2 × 0.3–0.4 mm, ovate-triangular, minutely verrucose, coma 1.5–2 cm.

Phenology. Collected in flower from January to December; in fruit from February to May.

Distribution. Brazil: Paraná, Rio de Janeiro, Santa Catarina, and São Paulo.

Oxypetalum cordifolium subsp. *pedicellatum* is found in low montane rainforest, montane rainforest, secondary rainforest, disturbed forest, grasslands, and along watercourses from 10 to 1050 m in Brazil. It is quite similar to *O. cordifolium* subsp. *brasiliense* in shape of the leaves, corona lobes, and pollinarium. It differs from the latter subspecies by its calyx lobe sinuses with a conspicuous fascicle of colleters (vs. no colleter or one colleter), caudicles shortly stipitate (vs. long-stipitate), and pollinia slightly curved (vs. pollinia clearly curved).

Specimens examined. BRAZIL. **Paraná:** Campina Grande do Sul, Rio Pardinho, *Hatschbach 7664* (MBM, RB). **Rio de Janeiro:** Parq. Nac. Itatiaia, *Occhioni 1211* (RB); Parati, Paraty à Cunha, *Nadruz et al. 473* (RB, SP); Rio de Janeiro, Parq. Nac. Tijuca, Morro da Cocanha, *Braga et al. 3926* (RUSU); Teresópolis, Parq. Nac. Serra dos Órgãos, *Occhioni 1257* (RB). **Santa Catarina:** Ribeirão, Tapera, *Klein et al. 7962* (HRB). **São Paulo:** Registro, *Fontella s.n.* (RB-145076).

6. Oxypetalum costae Occhioni, Dusenia 3(3): 200, tab. 11. 1952. TYPE: Brazil. Rio de Janeiro: "habitat Itatiaia, planalto 2000 m," 22 Nov. 1938, *F. Markgraf 3644 & A. C. Brade* (holotype, RB!). Figure 2.

Twining shrub, branches tomentose. Leaves with petiole 2.5–6.5 cm, tomentose with 3 or 5 colleters at blade base; blades 6–11 × 3–6 cm, ovate, camptobrochidodromous, basally cordate, apically acuminate, adaxially pubescent to tomentose, abaxially tomentose. Inflorescence 4- to 8-flowered, cymoid to corymbiform; peduncle ca. 1.3 cm, tomentose; pedicels 7–15 mm, pubescent. Floral buds 1.2–1.4 mm, ovate-triangular; calyx lobes 6–8 × 1.5–2 mm, with 1 or 2 colleters below each sinus within, linear-triangular or triangular, pubescent to tomentose outside, glabrous inside; corolla tube 3–3.2 mm, ± campanulate, puberulent outside, glabrous inside; lobes 12–15 × 3–3.5 mm, spreading, twisted, abaxially pubescent, adaxially with sparse trichomes; corona lobes 3–4 × 2–2.5 mm, ± rectangular, emarginate at undulate apex, adaxially with tooth extending slightly beyond the corona apex; gynostegium 3–3.2 mm high, ± sessile; anthers rectangular, with wings 1.5–2 mm, longer than dorsum; connective appendage 1–1.5 mm, ovate; corpusculum 0.70–0.80 × 0.28–0.30 mm, obovate, slightly longer than pollinia, thick, not laminar, planar; caudicles

0.16–0.18 mm, horizontal, with teeth indistinct and included; pollinia 0.60–0.69 × 0.20–0.27 mm, ± ovate or ± elliptical, slightly shorter than corpusculum; style-head 7–10 mm, elongate and exserted, bifid at apex with laminar branchlets. Fruit not seen.

Phenology. Collected in flower in November.

Distribution. Brazil: Rio de Janeiro.

Oxypetalum costae is endemic to Rio de Janeiro, growing in high-altitude grasslands at the edge of cloud forest, from 1800 to 2000 m in Brazil. It is quite similar to *O. insigne* var. *burchellii* (E. Fourn.) Fontella in indument, shape of the leaves, corona lobes, and laminar style-head. However, it differs from the latter variety, principally in the thick corpusculum that is not laminar and is slightly longer than the pollinia (vs. not thickened laminar corpusculum that is shorter than pollinia) and the teeth of the caudicles included (vs. teeth of the caudicles exserted).

Specimens examined. BRAZIL. **Rio de Janeiro:** Itatiaia, Planalto, *Markgraf 3644 & Brade* (RB); Parq. Nac. Itatiaia, Pico das Agulhas Negras, *Braga et al. 1570* (RB).

7. Oxypetalum glaziovianum Loes., Flora 72: 78. 1889. TYPE: Brazil. Rio de Janeiro: Feb. [as II] 1888, *A. F. M. Glaziou 17143* (lectotype, designated here, P!; isotypes, C not seen, K!, K photo at RB!).

Oxypetalum mourai Hoehne, Commiss. Linhas Telegr. Estratég. Matto Grosso Amazonas 38(1): 77, n. 30, pl. 8, fig. 2 et pl. 42. 1916. Syn. nov. TYPE: Brazil. Rio de Janeiro: Serra das Araras-RJ, [from Herb.] *L. Damazio 6060* ex herb. Schwacke [Herb. J. T. Moura] (lectotype, designated here, pl. 8, fig. 2, in Hoehne, 1916).

Twining vine, branches puberulent or sparsely covered with white, adpressed trichomes. Leaves with petiole 0.7–1.4 cm, absent or with 2 colleters at blade base; blades 6.7–7.5 × 1.2–1.4 cm, oblong or ± oblong, brochidodromous, basally cordate, apically acute-mucronate, adaxially and abaxially glabrous, midvein puberulent, trichomes sparse and scattered at margins. Inflorescence 3-flowered, sciadioidal; peduncle 1.6–2 cm, pubescent; pedicels 1–1.2 cm, pubescent. Floral buds ca. 6 mm, oblong; calyx lobes 1.8–2 × 0.5–0.6 mm, with 2 or 3 colleters below each sinus within, triangular, abaxially pubescent, adaxially glabrous; corolla shortly campanulate, tube 1.5–1.7 mm, outside and inside pubescent, at throat barbellate; lobes 8.5–8.8 × 1.2–1.5 mm, linear-oblong, spreading and slightly twisted, adaxially glabrous, abaxially pubescent; corona with lobes 1–1.3 × 0.8–1.2 mm, ± rotund or ± rhomboid, apex rounded, slightly undulate, inside with 2 lateral expansions; gynostegium ca. 4 mm high, ± sessile; anthers rectangular, with wings 0.5–0.6 mm, longer than the dorsum; connective appendage 0.6–0.7 mm, elliptical; corpusculum 2.44–3.11 × 0.29–0.52 mm, linear-oblong, thin, laminar, planar, 3× longer than pollinia; caudicles 0.26–0.35 mm, horizontal, with lateral teeth divergent, free, briefly or shortly stipitate; pollinia 0.66–0.82 × 0.29–0.35 mm, oblong or ± elliptical, shorter than corpusculum; style-head 2.8–3 mm, cylindrical, exserted, bifid from the middle or above, with lanceolate or linear branches. Follicle not seen.

Phenology. Collected in flower in January, February, and October.

Distribution. Brazil: Rio de Janeiro.

Oxypetalum glaziovianum is endemic to Rio de Janeiro, growing in Atlantic rainforest. According to Glaziou (1910: 461) this species also occurs at the crest of the Serra dos Órgãos, Teresópolis. *Oxypetalum glaziovianum* is quite similar to species in the *O. cordifolium* group in its flattened style-head and its laminar corpusculum; it is also quite similar to the *O. insigne* group in its flattened, laminar corpusculum. However, *O. glaziovianum* differs from these taxa principally in its spathulate or ± spathulate corolla lobes, the form of the corona ± rotund to ± rhomboid, and the divaricate branches of the style-head.

Lectotypification of *Oxypetalum glaziovianum* is necessary because there is no indication in the protologue as to where a holotype specimen was deposited. Of three isotype specimens distributed, *Glaziou 17143* (P) is selected.

The type of *Oxypetalum mourai* was not found in herbaria in Rio de Janeiro (R, RB), São Paulo (SP), or in various European herbaria (B, BM, K, M, P, W, WU). Plate 8, figure 2, in Hoehne (1916), based on *Damazio 6060*, ex herb. Schwacke (Herb. J. T. Moura), is selected as lectotype.

Specimens examined. BRAZIL. **Rio de Janeiro:** Teresópolis, Montanha do Louzada, *Saldanha 6894* (R); Serra Cavallo, *L. C. Brade 9839* (R).

8. Oxypetalum glaziovii (E. Fourn.) Fontella & Marquete, Bradea 9(5): 24. 2003. *Melinia glaziovii* E. Fourn. [as "glaziovi"], Fl. Bras. 6(4): 289. 1885. TYPE: Brazil. Rio de Janeiro: "in montibus Agulhas Negras," *A. F. M. Glaziou 8802* (lectotype, designated by Fontella-Pereira & Marquete, 2003: 24, P!; isotype, R!).

Melinia hilariana E. Fourn., Fl. Bras. 6(4): 290. 1885. TYPE: Brazil. Minas Gerais: *A. F. C. P. St.-Hilaire s.n.* (holotype, P!).

Oxypetalum urbanianum Silveira, Fl. Ser. Min.: 25. 1908. TYPE: Brazil. Minas Gerais: "in campis siccis in Serra do Papagaio, Apr. 1897," *A. Silveira 322* (holotype, R!, R photo at RB!).

Twining vine, branches puberulent. Leaves with petiole 5–13 mm, puberulent, with 2 colleters at blade base; blades 17–30 × 12–16 mm, ovate, brochidodromous, basally cordate, apically acuminate, adaxially and abaxially glabrous, puberulent only at margins and midnerve. Inflorescence 2- to 4-flowered, sciadioidal; peduncle 7–13 mm, puberulent; pedicels 3–5 mm, puberulent. Floral buds 4–5 mm, triangular or ovate-triangular; calyx lobes 2.2–3 × 0.4–0.5 mm, with 1 or 3 colleters below each sinus within, lanceolate, abaxially pubescent, adaxially glabrous, with verrucose trichomes 119–301 µm; corolla campanulate, tube 1–2 mm, glabrous outside, barbate inside, lobes 4–4.5 × 1.5–2 mm, ovate, reflexed, abaxially pubescent at middle, adaxially puberulent, with trichomes barbate at base with verrucose trichomes (68)116–179 µm; corona lobes 0.9–1 × 0.4–0.8 mm, oblong, adaxially with a conspicuous tooth oblong and fleshy; gynostegium 1–1.2 mm high, ± sessile; anthers quadrate, with wings 0.3–0.4 mm, longer than dorsum; connective appendage 0.3–0.5 mm, transverse-rectangular; corpusculum 0.26–0.29 × 0.07–0.08 mm, linear-oblong, thin, laminar, planar; caudicles 0.12–0.14 mm, horizontal, filiform, teeth included, shortly stipitate; pollinia 0.26–0.30 × 0.08–0.09 mm, linear-oblong, almost equal to corpusculum; style-head 2–2.2 mm, cylindrical, elongate and exserted, bifid or bilobate at apex. Follicle 2.8–3.5 × 4–7 cm, fusiform, with elongate-acuminate tip, puberulent; seeds not seen.

Phenology. Collected in flower from January to June and from September to December; in fruit from January to March and in December.

Distribution. Brazil: Minas Gerais (Fontella-Pereira & Marquete, 2003), Rio de Janeiro, and São Paulo.

Oxypetalum glaziovii is found in rocky thickets in high-altitude grasslands and campos rupestres, from 1800 to 2600 m in Brazil. This species is readily recognized by its small leaves, horizontal caudicles with included teeth, and a style-head that is elongated, exserted, and bifid at apex. It is morphologically similar to *O. pachyglossum* and *O. alpinum* var. *alpinum* in the caudicle with teeth included. It differs in its small ovate leaves (vs. long and narrowly oblong to lanceolate leaves in *O. pachyglossum* and *O. alpinum* var. *alpinum*). It is represented in Minas Gerais only by the type specimen for *Melinia hilariana*.

Specimens examined. BRAZIL. **Rio de Janeiro:** Prateleiras, Registro and Itamonte, Parq. Nac. Itatiaia, *Marquete et al. 451* (R, RB); Parq. Nac. Itatiaia, Abrigo Rebouças, *Castellanos 25670* (F, GUA, RB, SP); Resende, Serra da Mantiqueira, Mauá, Rancho, *Kaempfe 409* (RB). **São Paulo:** Queluz, Pedra da Mina, *Shepherd et al. 97-30* (HB, HRCB).

9. Oxypetalum insigne (Decne.) Malme, Ark. Bot. 21A(3): 31. 1927. *Calostigma insigne* Decne., Ann. Sci. Nat., Bot., ser. 2, 9: 344. 1838. TYPE: Brazil. São Paulo: "hab. in Brasilia prov. San-Paolo," *C. B. Gaudichaud 375* (holotype, P!; isotype, F!).

9a. Oxypetalum insigne (Decne.) Malme var. **insigne**

Calostigma guilleminianum Decne., Prodr. (DC.) 8: 581. 1844. TYPE: Brazil. Minas Gerais: 1834, *P. Claussen 354* (lectotype, designated here, P!; isotype, P fragment at F!). Figure 3.

Twining vine, branches tomentose. Leaves with petiole (3)9–15(31) mm, villous, with 2 or 3 colleters at blade base; blades (4)6–8(9.5) × (1.7)2.3–4(6) cm, ovate to obovate or elliptic to narrowly elliptic, brochidodromous, basally cordate, apically acuminate to mucronate, adaxially and abaxially densely pubescent to hirsute-tomentose. Inflorescence 3- to 10-flowered, cymoid to corymbiform; peduncle (0.3)0.5–1 cm, villous or woolly; pedicels 12–19(35) mm, tomentose to villous. Floral buds 1.5–2 cm, lanceolate to ovate; calyx lobes 4–6 × 0.4–0.5 mm, with 3 or 6 colleters below each sinus within, triangular-lanceolate, abaxially tomentose, adaxially glabrous, tomentose or puberulent, with verrucose trichomes 153–251 µm; corolla campanulate, tube 2.7–4.3 mm, pubescent outside, puberulent inside, lobes 14.5–40 × 1–4.2 mm, linear-lanceolate to linear-elongate, spiralled, reflexed, abaxially pubescent, adaxially puberulent, glabrous or glabrate, with verrucose trichomes 154–199 µm; corona lobes 3.5–4.5 × 1.2–1.5 mm, rectangular or oblong, emarginate or truncate, apex verrucose, tooth adaxially present, sometimes higher than corona lobe; gynostegium 1.7–3.5 mm high, sessile; anthers ± rectangular, with wings 0.7–1.3 mm, longer than dorsum; connective appendage 0.7–2.5 mm, ovate; corpusculum 1.6–2.0 × 0.2–0.5 mm, thin, laminar, planar, oblong, conspicuously longer than pollinia; caudicles 0.06–0.16 mm, horizontal, fixed at base of corpusculum, shortly pedicellate, teeth lateral, curved and free; pollinia 0.36–0.66 × 0.12–0.22 mm, oblong; style-head 3.8–8 mm, cylindrical, elongate and exserted, crateriform or coroniform, irregularly laciniate or bifid with trilobate apex. Fruit not seen.

Phenology. Collected in flower in February, from April to August, October, and December.

Distribution. Brazil: Minas Gerais, Rio de Janeiro, Santa Catarina, and São Paulo.

Oxypetalum insigne var. *insigne* is found in Rio de Janeiro in low montane and montane rainforest, secondary rainforest, thickets, gallery forest, and

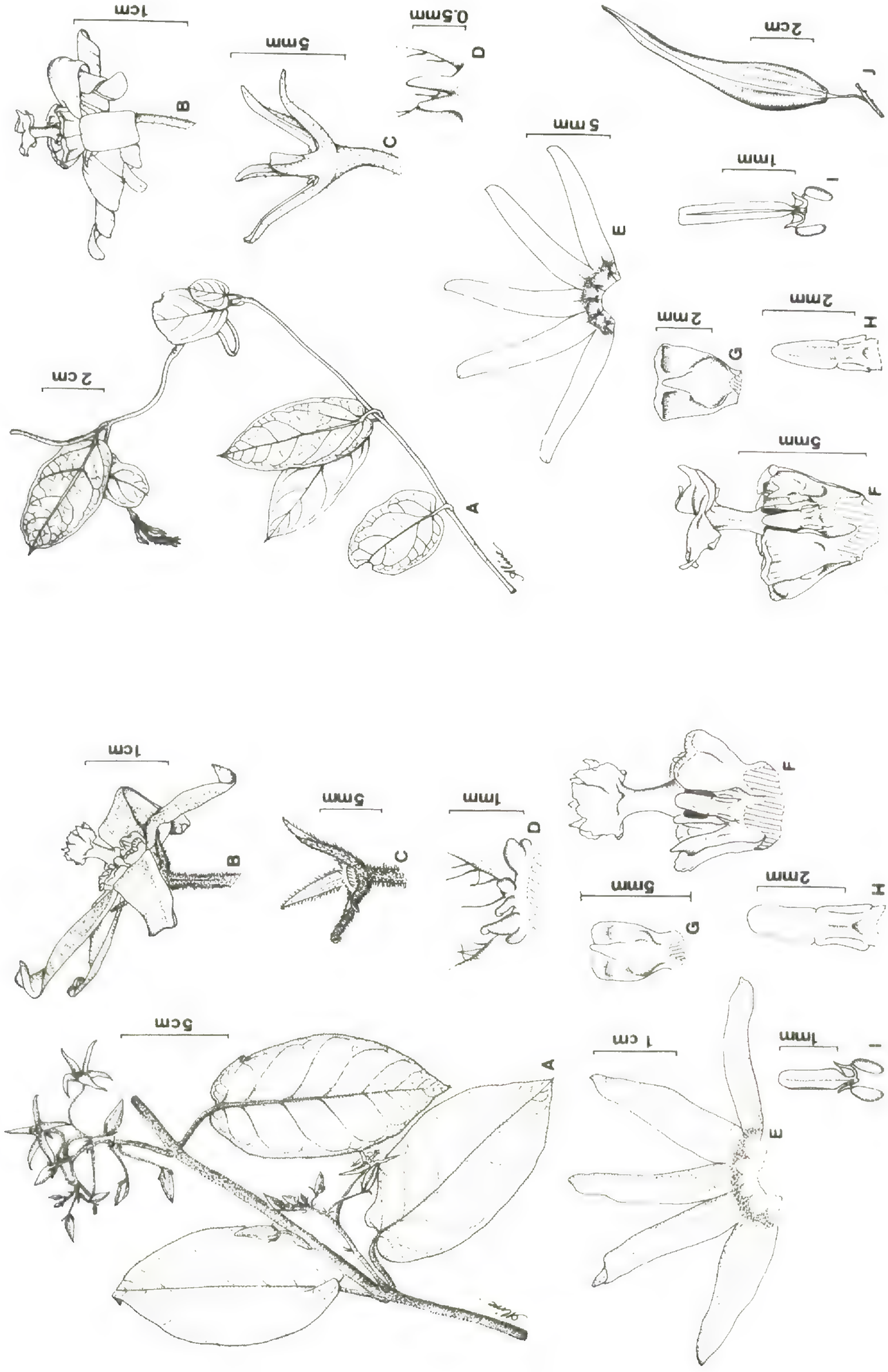


Figure 3 (left). *Oxypetalum insigne* var. *insigne*. —A. Flowering branch. —B. Flower. —C. Calyx. —D. Colleters in calyx lobe sinus. —E. Corolla lobes, adaxial view. —F. Corona and gynostegium with corolla lobes removed. —G. Corona lobe, adaxial view. —H. Anther, abaxial view. —I. Pollinarium. Drawn from *Brade 17472* by A. S. Lima.

Figure 4 (right). *Oxypetalum insigne* var. *glabrum*. —A. Flowering branch. —B. Flower. —C. Calyx. —D. Colleters in calyx lobe sinus. —E. Corolla lobes, adaxial view. —F. Corona and gynostegium with corolla lobes removed. —G. Corona lobe, adaxial view. —H. Anther, abaxial view. —I. Pollinarium. —J. Fruit. Drawn from *Rapini et al. 317* by A. S. Lima.

high-altitude grasslands. In other Brazilian states, *O. insigne* var. *insigne* occurs in campos rupestres, on marsh edges, and along watercourses and roadsides, from 50 to ca. 1800 m. It is quite similar to *O. rusticum* Rapini in the shape of the corolla and corona lobes, the laminar corpusculum, and a coroniform or crateriform style-head. However, it differs from this species principally in its densely pubescent to tomentose leaves (vs. pilose to villous leaves) and a shorter corpusculum (1.6–2 mm vs. 2.5–2.6 mm).

Lectotypification of *Calostigma guilleminianum* is necessary because there is no indication in the protologue as to where a holotype specimen was deposited. Of the two syntype specimens distributed, the specimen *Claussen 354* (P) is selected.

In addition to the taxa mentioned here, the following infraspecific taxa belonging to this group were also established: *O. insigne* var. *glaziovii* (E. Fourn.) Fontella & E. A. Schwarz (Fontella-Pereira & Schwarz, 1983), *O. insigne* var. *glabrum* (Decne.) Fontella & E. A. Schwarz (Fontella-Pereira & Schwarz, 1983), *O. insigne* f. *baetaeanum* (Silveira) Fontella & E. A. Schwarz (Fontella-Pereira & Schwarz, 1983), *O. insigne* var. *boraceiense* Fontella-Pereira & E. A. Schwarz (Fontella-Pereira & Schwarz, 1984b), *O. insigne* var. *burchellii* (E. Fourn.) Fontella (Fontella-Pereira, 1990), *O. insigne* var. *bresolinii* Fontella (Fontella-Pereira, 1990), and *O. insigne* var. *nitidum* (Malme) Fontella & E. A. Schwarz (Fontella-Pereira et al., 1997). *Oxypetalum insigne* var. *glabrum*, *O. insigne* f. *baetaeanum*, and *O. insigne* var. *insigne* are clearly delimited in the identification key by Fontella-Pereira et al. (1984). *Oxypetalum insigne* var. *insigne*, *O. insigne* var. *burchellii*, and *O. insigne* var. *bresolinii* were illustrated and clearly separated by Fontella-Pereira et al. (2004). *Oxypetalum insigne* var. *insigne*, *O. insigne* var. *glaziovii*, *O. insigne* var. *boraceiense*, *O. insigne* var. *burchellii*, and *O. insigne* var. *nitidum* were also clearly delimited by Farinaccio (2005).

Specimens examined. BRAZIL. **Minas Gerais:** *Guillemin s.n.* (P); Ouro Preto, Andorinhas Mariana–Belo Horizonte, Andorinhas, Belo Horizonte, *Fontella 1091* (RB); Camarinhas, *Marquete et al. 295* (RB); Lima Duarte, Dist. Conceição Ibitipoca, Ibitipoca to Santana de Garambel, *Marquete et al. 381* (R, RB); Dist. Conceição Ibitipoca, Parq. Est. Ibitipoca, Barro Preto Lagoa Seca, *Marquete et al. 342* (RB); Dist. Conceição Ibitipoca, Parq. Est. Ibitipoca, Monjolinho, *Marquete et al. 366* (RB); Dist. Conceição Ibitipoca, Parq. Est. Ibitipoca, Janela do Céu, *Marquete et al. 369* (RB). **Rio de Janeiro:** Itatiaia, Parq. Nac. Itatiaia, *Occhioni 1219* (RB); Maromba, *Brade 17472* (RB); Macieiras, *V. L. G. Klein et al. 666* (RB). **Santa Catarina:** Governador Celso Ramos, Jordão, *Klein 9581* (HBR). **São Paulo:** Cunha, Res. Flor., *Filho et al. 281* (HCRB, SP).

9b. Oxypetalum insigne var. **glabrum** (Decne.) Fontella & E. A. Schwarz, Bradea 4(3): 17. 1983. *Calostigma glabrum* Decne., Prodr. (DC.) 8: 581. 1844. *Oxypetalum glabrum* (Decne.) Malme, Ark. Bot. 21A(3): 29. 1927. *Oxypetalum glabrum* (Decne.) T. Mey., Dusenia 2(6): 366. 1951. TYPE: Brazil. Minas Gerais: "Sierra da Piedade," *P. Claussen 329* (holotype, P!, P photo at RB!). Figure 4.

Twining vine, branches glabrous or slightly pubescent. Leaves with petiole 6–7 mm, pubescent, with 2 colleters at blade base; blades 3.5–4.6 × 1.3–1.5 cm, elliptic, lanceolate, or ovate-lanceolate, brochidodromous, basally slightly cordate or rotundate, apically slightly acuminate, adaxially and abaxially glabrous or glabrescent, puberulent at nerves. Inflorescence 2- to 3-flowered, cymoid to corymbiform; peduncle 0.6–1.3 cm, puberulent; pedicels 11–14 mm, puberulent. Floral buds ca. 0.9 cm, ovate-lanceolate; calyx lobes 2.5–3.5 × 0.2–0.3 mm, with 1 or 2 colleters below each sinus within, triangular-linear, abaxially puberulent and adaxially glabrous, with smooth trichomes 61–99 μm; corolla campanulate, tube 1.8–2 mm, glabrous or glabrate outside and inside, lobes 8–13 × 1.8–2 mm, linear-lanceolate, spiralled, reflexed, abaxially glabrous or glabrate, adaxially glabrous; corona with lobes ± rectangular, emarginate or truncate, verrucose at apex, tooth adaxially present, usually equal or slightly shorter than corona lobe and with 2 fleshy processes; gynostegium ca. 2 mm high, sessile; anthers ± quadrate, with wings 1.8–2 mm, longer than dorsum; connective appendage 0.6–2.3 mm, ovate; corpusculum 1.53–1.83 × 0.19–0.22 mm, oblong, thin, laminar, planar, conspicuously longer than pollinia; caudicles 0.21–0.22 mm, horizontal, fixed at base of corpusculum, shortly pedicellate; teeth lateral, curved and free; pollinia 0.36–0.43 × 0.11–0.12 mm, oblong; style-head 2.8–4 mm, cylindrical, elongate and exserted, apex planar, shell-shaped or bifid, with divaricate or erect branches; 1 vascular bundle. Follicles 7 × 1.5 cm, lanceolate, with long-acuminate tip, glabrous; seed not seen.

Phenology. Collected in flower in January, April, May, November, and December; in fruit in May.

Distribution. Brazil: Minas Gerais, Rio de Janeiro, and São Paulo.

Oxypetalum insigne var. *glabrum* is found in Rio de Janeiro in montane rainforest; in other Brazilian states, it also occurs in grasslands and campos rupestres from 1250 to 1752 m. It is morphologically similar to *O. insigne* var. *insigne* or *O. insigne* var. *glaziovii* in the shape of the corolla and corona lobes, the laminar corpusculum, and the style-head. However, it differs from *O. insigne* var. *insigne* principally

in its corolla lobes adaxially glabrous or glabrate (vs. sparsely pubescent), the narrower corpusculum (0.19–0.22 mm vs. to 0.2–0.5 mm), and the shorter style-head (2.8–4 mm vs. 3.8–8 mm) that is bifid and shell-shaped at the apex (vs. trilobate at apex).

Specimens examined. BRAZIL. **Minas Gerais:** Santa Barbara, Serra do Caraça, Pico do Inficionado, *Rapini et al. 317* (SP, SPF). **Rio de Janeiro:** Teresópolis, *Glaziou 1770* (R).

9c. Oxypetalum insigne var. **glaziovii** (E. Fourn.) Fontella & E. A. Schwarz, Bradea 4(3): 17. 1983. *Calostigma glaziovii* E. Fourn., Fl. Bras. 6(4): 286, pl. 81. 1885. *Calostigma glabrum* var. *glaziovii* (E. Fourn.) Hoehne, Commiss. Linhas Telegr. Estratég. Matto Grosso Amazonas, 38(2): 16, tab. 6, fig. 1, et pl. 7. 1916. *Oxypetalum glabrum* var. *glaziovii* (E. Fourn.) T. Mey., Dusenia 2(6): 368. 1951. TYPE: Brazil. Rio de Janeiro: Friburgo, *A. F. M. Glaziou 14087* (holotype, P!, P photo at HB!; isotype, R!).

Oxypetalum kalanthum Silveira, Fl. Serr. Min.: 25. 1908. TYPE: Brazil. Minas Gerais: "in campis ad Morro do Bispo, prope urbem Ayuruoca," Nov. 1897, *A. Silveira 318* (holotype, R!; isotypes, B, B photo at F, RB!, NY!).

Calostigma glabrum var. *macranthum* Hoehne, Commiss. Linhas Telegr. Estratég. Matto Grosso Amazonas 38(2): 17. 1916. *Oxypetalum glabrum* var. *macranthum* (Hoehne) T. Mey., Dusenia 2(6): 366. 1951. TYPE: Brazil. Rio de Janeiro: Alto da Serra do Itatiaia, Oct. 1903, *P. Dusén 2002* (holotype, R!).

Twining vine, branches pubescent or glabrescent. Leaves with petiole 0.5–1 cm, tomentose or pubescent, with 1 or 2 colleters at blade base; blades 4–6.5 × 1.7–2.2 cm, ovate or ovate-lanceolate, brochidodromous, basally cordate or rotundate, apically acuminate, adaxially and abaxially glabrous or puberulent at midnerve and at margin. Inflorescence 2- to 4-flowered, cymoid to corymbiform; peduncle 0.4–1 cm, pubescent or slightly pubescent; pedicels 8–10 mm, pubescent. Floral buds 0.8–0.9 cm, triangular; calyx lobes 3.8–5.2 mm, with 3 or 4 colleters below each sinus within, triangular-lanceolate, abaxially slightly pubescent, adaxially glabrous, with verrucose trichomes 92–140 µm; corolla campanulate, tube 1.8–2 mm, glabrous outside, pubescent inside, lobes 11–16 × 2–2.5 mm, lanceolate to elongate, twisted, reflexed or spreading, abaxially puberulent, adaxially sparsely pubescent, with verrucose trichomes 75–117 µm; corona lobes 1–1.5 × 1.2–1.8 mm, rectangular, with emarginate or truncate, verrucose apex, tooth adaxially present, equal or slightly shorter than corona lobe with 2 fleshy processes; gynostegium 1.8–2 mm high, sessile; anthers ± rhombic, with wings 1.8–1.9 mm, longer than dorsum; connective appendage 0.7–2.4 mm, ovate; corpusculum 1.23–1.35 × 0.15–0.21 mm, oblong, thin, laminar, planar, conspicuously longer than pollinia; caudicles 0.21–0.23 mm, horizontal, fixed on base of corpusculum, shortly stipitate, teeth lateral, curved and free; pollinia 0.36–0.39 × 0.13–0.16 mm, oblong; style-head 2.7–3.8 mm, cylindrical, elongate and exserted, usually planar with 2 trilobate branches at apex. Fruit not seen.

Phenology. Collected in flower in January, February, June, August, and September to December.

Distribution. Brazil: Minas Gerais, Rio de Janeiro, and São Paulo.

Oxypetalum insigne var. *glaziovii* is found in montane rainforest and high-altitude grasslands from 1700 to 2550 m in Brazil. It is quite similar to *O. insigne* var. *glabrum* in the shape of the leaves, corolla and corona lobes, and pollinarium. It differs from *O. insigne* var. *glabrum* principally in its corolla lobes adaxially sparsely pubescent (vs. glabrous or glabrate), corpusculum truncate at apex and shorter (1.23–1.35 mm vs. not truncate, 1.53–1.83 mm), and style-head cylindrical, usually with trilobate branches at apex (vs. shell-shaped or bifid at apex).

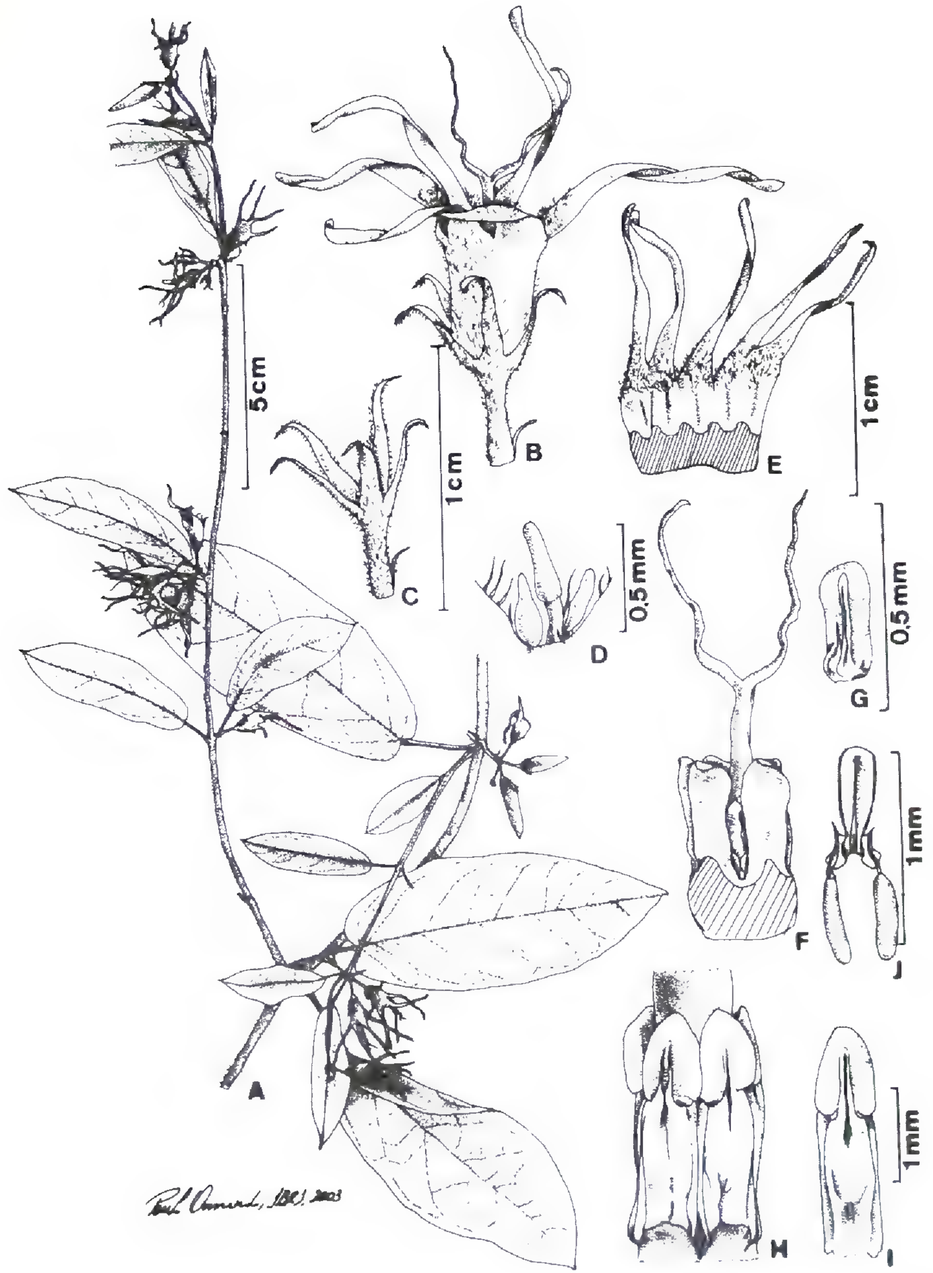
Specimens examined. BRAZIL. **Minas Gerais:** Caparaó, Parq. Nac. Caparaó, *Hatschbach 31432 & Ahumada* (S). **Rio de Janeiro:** Itatiaia, Prateleiras, Registro and Itamonte, Hotel Alsene, *Marquete et al. 450* (R, RB); Macaé, Dist. Glicério, Pico do Frade, *Ribeiro et al. 914* (RB); Petrópolis, Caxambú, Morro do Inferno, *Fernandes 469* (GUA); Teresópolis, Pedra Chapadão, *Brade 9960* (R). **São Paulo:** Boracéa, Salesópolis, *Kuhlmann 1694* (SP).

10. Oxypetalum jacobinae Decne., Prodr. (DC.) 8: 584. 1844. *Gothofreda jacobinae* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Bahia: "ad Jacobina," *J. S. Blanchet 3646* (holotype, G!, G photo at NY!, P!, RB!). Figure 5.

Oxypetalum dentatum E. Fourn., Fl. Bras. 6(4): 263. 1885. *Gothofreda dentata* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Minas Gerais: "ad Capoeiras pr. Facenda de Monte Novo," *A. F. C. P. St.-Hilaire 1077* (holotype, P!; isotype, P fragment at F!, P photo at F!).

Oxypetalum lagoense E. Fourn., Fl. Bras. 6(4): 264. 1885. *Gothofreda lagoensis* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Minas Gerais: Lagoa Santa, "in marginibus silvarum," *Hb. Eug. Warming 1864/2* (holotype, C!).

Twining vine, branches tomentose. Leaves with petiole 0.5–1.5 cm, pubescent or tomentose; blades 5–10 × 1.5–4 cm, moss green adaxially, greenish abaxially, ovate-oblong, camptobrochidodromous, apically acuminate, base cordate or auriculate, adaxially pubescent or tomentose, abaxially densely pubescent



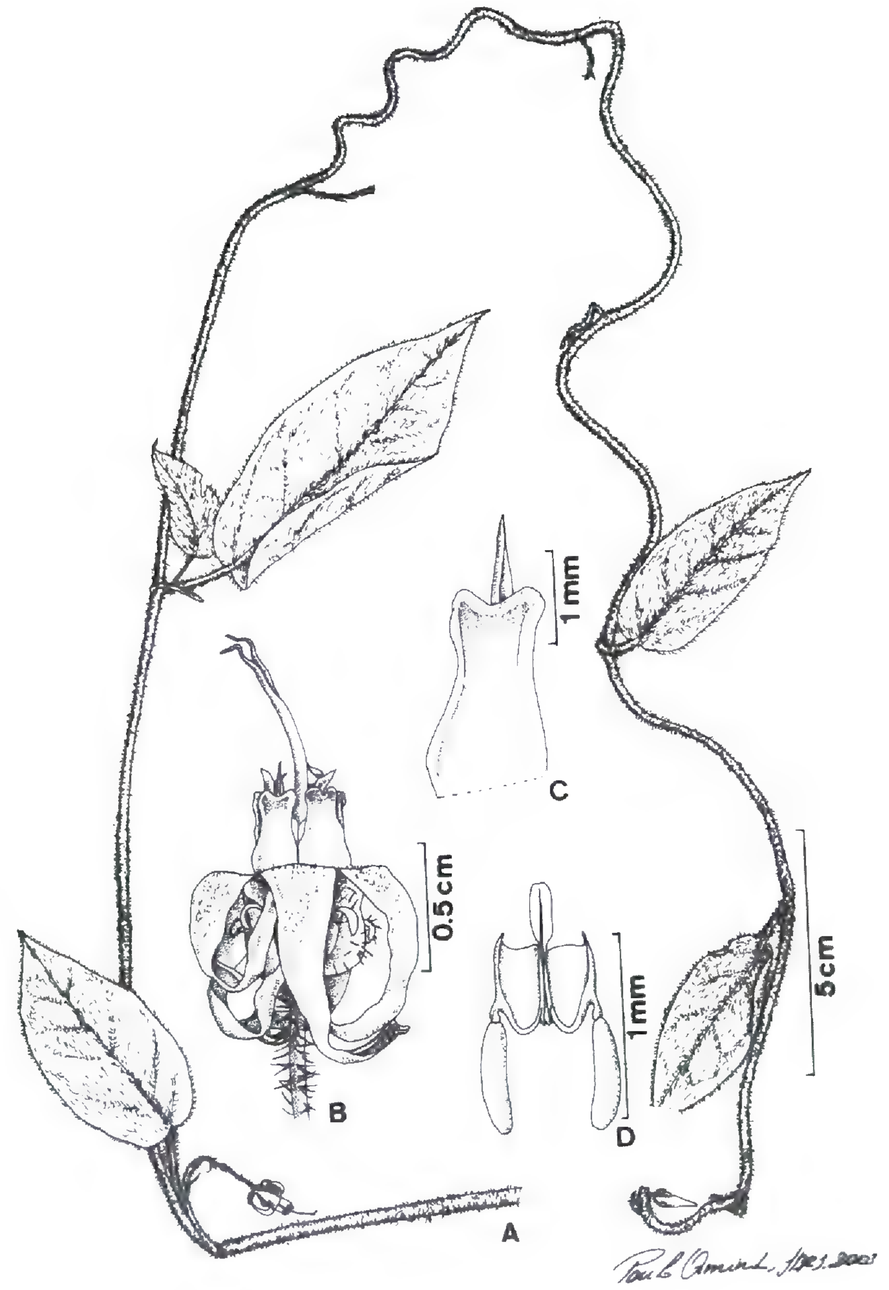


Figure 5 (left). *Oxypetalum jacobinae*. —A. Flowering branch. —B. Flower. —C. Calyx. —D. Colleters in calyx lobe sinus. —E. Corolla lobes, adaxial view. —F. Corona and gynostegium with corolla lobes removed. —G. Corona lobe, adaxial view. —H. Gynostegium part. —I. Anther, abaxial view. —J. Pollinarium. Drawn from *Fontella 1023, W. Vidal 362 e M. Vidal et al. 392*. Drawings by P. Ormindo.

Figure 6 (right). *Oxypetalum lutescens*. —A. Flowering branch. —B. Flower. —C. Corona lobe, abaxial view. —D. Pollinarium. A–C drawn from *Glaziou 6903*, D drawn from Hoehne 1916, pl. 9, fig. 1. Drawings by P. Ormindo.

or tomentose. Inflorescence 5- to 8-flowered, sciadioidal, ± sessile, flowers 1–1.5 cm; pedicels 2–3.5 mm, tomentose. Floral buds 1.4–1.5 cm, triangular; calyx lobes 5–5.5 × 0.5–1 mm, with 1 or 3 colleters below each sinus within, linear, abaxially hirsute, adaxially pubescent, verrucose trichomes 260–447 µm; corolla tube 4–6 mm, ± campanulate, pubescent outside, pubescent or tomentose inside, barbellate at throat, lobes 7–12 × 1.5–2 mm, linear, ± erect, twisted, abaxially and adaxially pubescent or glabrescent, verrucose trichomes 165–280 µm; corona lobes 3–3.5 × 1–1.5 mm, ± rectangular or ± oblong, apex obtuse or rounded, adaxially with linear tooth usually reaching to tip of corona lobe; gynostegium 2–3 mm high, ± sessile; anthers rectangular, with wings 2.5–3 mm, longer than dorsum; connective appendage 0.5–0.6 mm, ovate-acuminate; corpusculum 0.60–0.65 × 0.25–0.27 mm, ± oblong, thin, not laminar, planar, longer than pollinia; caudicles 0.26–0.28 mm, fixed at base of corpusculum, ± horizontal, shortly stipitate, teeth lateral, curved and free; pollinia 0.45–0.53 × 0.13–0.15 mm, apex oblong or obtuse, base rounded, slightly shorter than corpusculum; style-head 9–11 mm, ± cylindrical, elongate and exserted, bifid to middle, branches linear. Fruit not seen.

Phenology. Collected in flower in April, May, and October.

Distribution. Brazil: Bahia, Minas Gerais, and Rio de Janeiro.

Oxypetalum jacobinae is found in Brazil in low montane or montane rainforest, semi-deciduous seasonal forest, secondary rainforest, thickets, grasslands, and along roadsides, from 200 to 1250 m. It is quite similar to *O. erianthum* Decne. in habit and pubescence, the ± campanulate corolla, and the rectangular anthers. It differs from *O. erianthum* principally in its oblong to ± rectangular corona lobes with apex obtuse or rounded (vs. oval corona lobes with apex rounded or truncate), ± horizontal caudicles with free teeth (vs. descending caudicles with included teeth), and ± oblong and broader corpusculum (vs. linear-oblong and narrowed corpusculum).

Specimens examined. BRAZIL. **Bahia:** Jacobina, *Blanchet 3646* (P). **Minas Gerais:** Viçosa, Prefeitura, Inst. de Ciências Biol. Univ. de Viçosa, *Fontella 1058 & Vidal* (RB). **Rio de Janeiro:** Teresópolis, Serra de Teresópolis, *Neves Armond s.n* (R-5146).

11. Oxypetalum lanatum Decne. ex E. Fourn., Fl. Bras. 6(4): 266, pl. 76. 1885. TYPE: Brazil. Rio de Janeiro: "in summo jugo Serra dos Órgãos, aprili," *A. F. M. Glaziou 4093* (lectotype, designated here, P!. P photo at HB!; isotypes, K!, NY!, R!).

Twining vine, branches lanose. Leaves with petiole 5–10 mm, fulvous-tomentose, with 2 colleters at blade base; blades 4–5 × 1.5–2 cm, ovate or oblong-ovate, apically acute or mucronate, basally slightly cordate, camptobrochidodromous, adaxially tomentose, abaxially fulvous-woolly. Inflorescence (2)4- to 5-flowered, cymoid to corymbiform; peduncle 6–10 mm, fulvous-tomentose; pedicels 10–20 mm, tomentose. Floral buds 0.8–1.2 cm, elliptical; calyx lobes 5–6 × 1.5–1.8 mm, with 1 or 2 colleters below each sinus within, linear-lanceolate, abaxially tomentose, adaxially pubescent, with verrucose trichomes 236–472 µm; corolla tube 3–4.5 mm, campanulate, pubescent outside, glabrous inside, lobes 10–12 × 5–6 mm, base vinaceous, lanceolate or ± elliptical, spreading, twisted, abaxially pubescent, adaxially glabrous, with verrucose trichomes; corona 3–4.5 × 1.5–2 mm, rectangular, apex truncate, tooth adaxially present, longer than corona lobe; gynostegium 3–3.2 mm high, ± sessile; anthers ± rectangular, with wings 1.5–1.7 mm, slightly longer than dorsum; connective appendage 1–1.5 mm, ovate; corpusculum 1.10–1.20 × 0.30–0.35 mm, linear or oblong, thin, laminar, planar, conspicuously longer than pollinia; caudicles 0.36–0.38 mm, not fixed on base of corpusculum, horizontal, teeth lateral, curved and free, shortly pedicellate; pollinia 0.50–0.54 × 0.15–0.17 mm, oblong, slightly curvate, 2× shorter than corpusculum; style-head 6–7 mm, cylindrical, exserted, bifid to middle, with divaricate branchlets. Follicle 6.2–8.7 × 1.2–1.7 cm, lanceolate or fusiform with acuminate tip, base obtuse, densely tomentose; seed not seen.

Phenology. Collected in flower from January to June, September, and November; in fruit April, May, August, September, and November.

Distribution. Brazil: Minas Gerais and Rio de Janeiro.

Oxypetalum lanatum is found in montane rainforest, gallery forest, cloud forest, high-altitude grasslands, and campos rupestres on rocky outcrops from 1000 to 2400 m in Brazil. It is quite similar to *O. sublanatum* in habit, shape, and indumentum of the leaf blade, flowers, corona lobes, and laminar corpusculum. It differs from this taxon principally in its widely spreading corolla lobes (vs. reflexed corolla lobes), corona lobes adaxially with a conspicuous tooth surpassing its apex (vs. tooth absent), and the elongate, bifid, and exserted style-head (vs. style-head shorter and ± entire, enclosed by corona at apex).

Lectotypification of *Oxypetalum lanatum* is necessary because there is no indication in the protologue as to where a holotype specimen was deposited. Of the four syntype specimens distributed, *Gardner 5811* (CAMB, K, P, R), *Glaziou 7759* (P), *Tweedie s.n.* (K),

and *Glaziou 4093* (P), the *Glaziou 4093* specimen is selected as lectotype.

Specimens examined. BRAZIL. **Minas Gerais:** Lima Duarte, Conceição de Ibitipoca, Parq. Est. Ibitipoca, Morro da Lombada, *Marquete et al. 353* (RB); Conceição de Ibitipoca, Parq. Est. Ibitipoca, to Lombada, *Marquete et al. 354* (R, RB); Conceição de Ibitipoca, Parq. Est. Ibitipoca, Lagoa Seca, Brejo Alto, *Marquete et al. 347* (RB); Conceição de Ibitipoca, Parq. Est. Ibitipoca, Barro Preto, *Marquete et al. 367* (RB); Conceição de Ibitipoca, Parq. Est. Ibitipoca, Barro Preto, *Marquete et al. 368* (R, RB). **Rio de Janeiro:** Nova Friburgo, *Edmundo 43* (RB); Petrópolis, Correas, Açú, Morro da Bandeira, *Farney et al. 804* (RB); Santa Maria Madalena, Parq. Est. Desengano, Pedra do Desengano, *Martinelli et al. 13251* (RB); Teresópolis, Serra dos Órgãos, Rio Soberbo, *Glaziou 4093* (NY, R); Serra dos Órgãos, *Gardner 5811* (CAMB, K, P, R); *Glaziou 7759* (P); *Luetzelburg 6545* (P); Serra dos Órgãos-Morro Assu, *Luetzelburg 6567* (P). Sine loc.: in Brasilia australi, *Tweedie s.n.* (K).

12. Oxypetalum lutescens E. Fourn., Fl. Bras. (6)4: 273. 1885. *Gothofreda lutescens* (E. Fourn) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Rio de Janeiro: Nova Friburgo, Jan. 1874, *A. F. M. Glaziou 6903* (holotype, P!; isotypes, K!, R!, K photo at RB!, HB!). Figure 6.

Twining vine, branches gold-villose. Leaves with petiole 0.5–2.5 cm, villose, with 2 colleters at blade base; blades 5–9 × 1.8–3.2 cm, ovate-lanceolate or ovate, apically acuminate, basally truncate or ± cordate, adaxially slightly villose, abaxially villose. Inflorescence 1-flowered, cymoid to corymbiform; peduncle ca. 1 cm, villose; pedicels 1–1.5 cm, villose. Floral buds ca. 0.8 cm, conical; calyx lobes 5–7 × 0.5–1 mm, with 1 colleter below each sinus within, linear-triangular, abaxially villose; corolla tube 3–4 mm, campanulate, slightly pubescent outside, glabrous inside, glabrous at throat, lobes 8–15 × 2–2.5 mm, linear, spiralled, reflexed, abaxially pubescent, adaxially glabrous or ± glabrous; corona 2.5–2.7 × 1.5–1.7 mm, rectangular, exserted, apex emarginate, tooth adaxially present, filiform, surpassing to tip of corona lobe; gynostegium 1.3–1.5 mm high, ± sessile; anthers ± triangular, with wings 1–1.5 mm, longer than dorsum; connective appendage 1–1.3 mm, ovate-acuminate; corpusculum 0.58–0.64 × 0.09–0.11 mm, linear, as long as pollinia, thin, not laminar, planar; caudicles 0.30–0.34 mm, fixed up to middle of corpusculum, horizontal, teeth lateral, curved, free; pollinia 0.60–0.65 × 0.23–0.25 mm, cylindrical; style-head 9.5–10 mm, elongate and exserted, bifid at apex with filiform branches. Fruit not seen.

Phenology. Collected in flower in January.

Distribution. Brazil: Rio de Janeiro.

Oxypetalum lutescens is endemic to Rio de Janeiro, growing in Atlantic rainforest. It is quite different from other species in *Oxypetalum* by its leaves that are villous to slightly villous, the reflexed corolla lobes, the adaxial corona lobes with tooth surpassing its apex, the caudicles broad and inserted along the corpusculum and above the middle, and the filiform style-head elongated and exserted. It is similar to the *O. insigne* group and to *O. lanatum*, *O. schottii*, and *O. regnellii* in its horizontal and broad caudicles. It differs from these taxa by the style-head elongate and exserted with filiform branches, the tooth of the corona lobes clearly surpassing the apex of the lobes, and the broad caudicles fixed up to the middle of the corpusculum. There is only one collection, which was made by Glaziou in 1874 (*Glaziou 6903*) and is kept in the herbaria P and R. Modern collecting efforts have not revealed extant populations.

Specimen examined. BRAZIL. **Rio de Janeiro.** Nova Friburgo, ao Alto, *Glaziou 6903* (R).

13. Oxypetalum molle Hook. & Arn., J. Bot. 1: 289. 1834. *Gothofreda mollis* (Hook. & Arn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Santa Catarina: *J. Tweedie 231* (holotype, K!, K photo at RB!).

Oxypetalum nigrescens E. Fourn., Fl. Bras. 6(4): 264. 1885. *Gothofreda nigrescens* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. São Paulo: "in humidis," *L. Riedel 1819* (holotype, LE not seen).

Oxypetalum suaveolens E. Fourn., Fl. Bras. 6(4): 264, pl. 75. 1885. *Gothofreda suaveolens* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Minas Gerais: "Lagoa Santa," *E. Warming s.n.* (holotype, P!; isotype, C!).

Oxypetalum oliganthum Malme, Kongl. Svenska Vet.-Akad. Handl. 34(7): 46. 1900. TYPE: Brazil. Rio Grande do Sul: "Colonia Ijuhy, inter oppida Santo Angelo e Cruz Alta, 4 Apr. 1893," *G. O. A. Malme 754* (holotype, S not seen; isotype, R!).

Oxypetalum campanulatum Hand.-Mazz., Denkschr. Akad. Wiss. Wien, Math.-Nat. 79(2): 381. 1931. TYPE: Brazil. "Apiahy, Feb. 1884," *Puiggari s.n* (holotype, W!).

Twining vine, branches tomentose or velvety. Leaves with petiole 0.9–2(3.3) cm, velvety, with 3 or 5 colleters at blade base; blades 4.5–8.5 × 2.3–3.4 cm, oval-lanceolate, camptobrochidodromous, basally cordate to truncate, apically acuminate, adaxially tomentose, abaxially velvety. Inflorescence 2- to 7-flowered, cymoid to corymbiform; peduncle 0.2–2(6) cm, tomentose to velvety; pedicels 14–24 mm, tomentose to velvety. Floral buds 0.9–1.2 cm, triangular; calyx lobes 7–10 × 1–3 mm, with 3 or 4 colleters below each sinus within, triangular to linear-lanceolate, adaxially tomentose, abaxially puberulent or pubescent, with smooth trichomes 122–197 µm;

corolla tube 3.4–6.8 mm, campanulate, pubescent outside, glabrous inside, lobes 9–14 × 3.8–6 mm, ovate-oblong or ovate-lanceolate, spreading, twisted, abaxially pubescent to puberulent, with smooth trichomes, adaxially verrucose to papillose-verrucose; corona 3.5–4.5 × 1.7–2.8 mm, oblong to rectangular, apex emarginate to truncate, verrucose, tooth adaxially present, higher than corona lobe, with 2 fleshy processes at base; gynostegium 1.3–1.8 mm high, ± sessile; anther ± rhombic, with wings 0.7–1.2 mm, longer than dorsum; connective appendage 1.8–2 mm, oblong; corpusculum 1.16–1.42 × 0.28–0.42 mm, oblong, narrow at base, thin, laminar, planar; caudicles 0.06–0.16 mm, fixed on base of corpusculum, horizontal, shortly pedicellate, teeth lateral, curved, free; pollinia 0.40–0.56 × 0.14–0.18 mm, oblong, ellipsoid-oblong; style-head 4.8–5.5 mm, cylindrical, elongate and exserted, thick, bifid from the middle, branches 2.8–3 mm, thick, slightly trilobed. Fruit not seen.

Phenology. Collected in flower in February, March, from May to August, October, and November.

Distribution. Brazil: Goiás, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo; Argentina.

Oxypetalum molle is found in low montane and montane rainforest, disturbed forest, secondary rainforest, seasonal forest, rainforest with *Araucaria*, and in cerrado and cerradão from 150 to 990 m in Brazil. It is quite similar to *O. lanatum* in habit, shape of the corona lobes, and laminar corpusculum. However, it differs from that taxon principally in its corolla lobes adaxially papillose to verrucose and corpusculum almost 3× longer than the pollinia (vs. corolla lobes adaxially glabrous and corpusculum shorter than the pollinia).

Specimens examined. BRAZIL. **Goiás:** Campos Belos, Taguatinga, *Rizzo & Barbosa 3784* (RB). **Minas Gerais:** Viçosa, São Miguel, *Mexia 4765-a* (S). **Paraná:** Manoel Ribas, *Hatschbach 33501* (INPA). **Rio de Janeiro:** Petrópolis, Sertão de Carangola, *Marquete et al. 285* (R, RB). **Santa Catarina:** Naufragrados, *Klein & Bresolin 9213* (HBR, RB). **São Paulo:** Limeira, Fazenda Morro Azul, forest of S.A.F.B., *Kuhlmann 688* (US).

14. Oxypetalum pachyglossum Decne., Prodr. (DC.) 8: 585. 1844. *Gothofreda pachyglossa* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. São Paulo: *C. B. Gaudichaud 389* (holotype, P!).

Oxypetalum coriaceum Decne., Prodr. (DC.) 8: 585. 1844. *Gothofreda coriacea* (Decne.) Kuntze, Revis. Gen. Pl. 2: 419. 1891. TYPE: Brazil. *C. B. Gaudichaud 388* (holotype, P!).

Oxypetalum densiflorum Decne., Prodr. (DC.) 8: 584. 1844. *Gothofreda densiflora* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Bahia: "humidis," *P. Salzmann 328* (holotype, P!).

Oxypetalum paludosum Decne., Prodr. (DC.) 8: 585. 1844. *Gothofreda paludosa* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Bahia: "in paludosis," *J. S. Blanchet 2017* (holotype, BM!, BM photo at RB!).

Oxypetalum selloanum E. Fourn., Fl. Bras. 6(4): 260. 1885. *Gothofreda selloana* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Bahia: *F. Sellow s.n.* (lectotype, designated here, F!).

Oxypetalum luschnathii E. Fourn., Fl. Bras. 6(4): 261. 1885. *Gothofreda luschnathii* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 419. 1891. TYPE: Brazil. Bahia: "ad Cruz de Casme," *B. Luschnath 235* (holotype, LE not seen, LE photo at RB!).

Oxypetalum villosum E. Fourn., Fl. Bras. 6(4): 262. 1885. Syn. nov. *Gothofreda villosa* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Serra Mantiqueira: *J. E. B. Pohl 3766* (holotype, W!, W photo at RB!).

Cystostemma glandulosum Silveira, Fl. Serr. Min. 10: tab. 1. 1908. TYPE: Brazil. Minas Gerais: "prope Aguas Virtuosas," Oct. 1899, *A. Silveira 285* [Hb. Silveira] (lectotype, designated here, R!).

Twining vine, branches pubescent to glabrous. Leaves with petiole 7.5–20.5 mm, pubescent, with 4 colleters at blade base; blades 5–15 × 0.7–4.2 cm, oblong to oblong-lanceolate or oblong to lanceolate, brochidodromous, basally cordate, apically acuminate, abaxially usually with conspicuous nerves, abaxially and adaxially glabrous with trichomes at base, near the midnerves. Inflorescence 10- to 13-flowered, dichasially sciadioidal-umbelliform; peduncle 16–53(81) mm, pubescent; pedicels 10.6–12 mm, tomentose. Floral buds 0.3–0.4 cm, ovate; calyx lobes 2.5–3 × 0.7–1 mm, with 2 or 4 colleters below each sinus within, triangular-lanceolate, abaxially pubescent, adaxially glabrous, with smooth trichomes 65–148 µm; corolla rotate, tube 2–3 mm, pubescent outside, barbellate inside, lobes 4–4.5 × 2.5 mm, oval-lanceolate, widely spread, abaxially and adaxially glabrous; corona 2.5–3 × 1.5–2 mm, oblong, lobes with profoundly emarginate apex, with 2 linear lateral appendages, slightly shorter than corona; gynostegium 1.8–2 mm high, ± sessile; anthers rectangular, with wings 0.6–0.8 mm, longer than dorsum; connective appendage 0.7–0.9 mm, ovate; corpusculum 0.48–0.64 × 0.16–0.18 mm, planar, oblong-lanceolate or lanceolate; caudicles 0.14–0.22 mm, descending, fixed on base of corpusculum, shortly stipitate, teeth included; pollinia 0.62–0.7 × 0.20–0.22 mm, oblong to ovate, longer than corpusculum; style-head 0.7–0.8 mm, conical, slightly emarginate, shortly exserted, usually included, covered by connective appendages. Follicle 6.8–9 × 0.7–1 cm, fusiform, with acuminate tip,

pubescent; seeds ovate or oblong, slightly curved, slightly verrucose, 5.5–6 × 2.8–3.5 mm, coma 8–9 mm.

Phenology. Collected in flower from January to May, July, and November; in fruit in January, March, August, and November.

Distribution. Brazil: Bahia, Mato Grosso do Sul, Minas Gerais, Paraná, Rio de Janeiro, and São Paulo.

Oxypetalum pachyglossum is found in marshes and very wet areas of low montane and montane rainforest, seasonal forest, secondary forest, cerrado, gallery forest, grasslands, campos rupestres, and high-altitude grasslands from 600 to 2000 m in Brazil. It is quite similar to *O. alpinum* in the shape of the leaves and flowers, especially the corona lobes and pollinarium. It differs from this taxon principally in its rotate corolla with the tube adaxially barbellate at throat (vs. a campanulate corolla with the tube adaxially glabrous at throat), corona lobes usually enclosing the gynostegium (vs. corona lobes not enclosing the gynostegium), and the shorter style-head (vs. a longer style-head).

Fontella-Pereira mentioned lectotypification for *Oxypetalum pachyglossum* in the "Resumos XXXVIII Congresso Nacional de Botânica" (1987: 129), referring to *Gaudichaud 389* (P) as lectotype. However, this ephemeral publication is not advised by Recommendation 30A.1 in the *International Code of Botanical Nomenclature* (McNeil et al., 2006), and for this reason we designate the lectotype here. There was no indication in the protologue (Decaisne, 1844) as to where a holotype specimen was deposited. Of the two syntype specimens distributed, the *Gaudichaud 389* (P) specimen is selected.

Lectotypification of *Cystostemma glandulosum* is necessary because there is no indication in the protologue as to where a holotype specimen was deposited. Of the three syntypes examined, the specimen collected by Alvaro Silveira (ex Herbario Silveira 285) in October 1899, is selected.

The name *Oxypetalum selloanum* was described by Fournier in Martius (1885) and transferred to *Gothofreda* (nom. rej.) by Kuntze in 1891. The only specimen mentioned by Fournier was "Ad Bahia: Sello," and this specimen was found neither in Berlin (B) nor in Paris (P). Therefore, a duplicate of this specimen, *F. Sello s.n.* (F), is selected as lectotype.

Decaisne mentioned the specimens *Blanchet 14* and *Blanchet 2017* in his 1844 protologue of *Oxypetalum paludosum*. A specimen of *Blanchet s.n.* was found in Paris (P), and *Blanchet 2017* was found at the British Museum in London (BM). Therefore, no type is needed or selected here.

Specimens examined. BRAZIL. **Bahia:** Porto Seguro, Porto Seguro and Santa Cruz de Cabrália, Porto Seguro, *Mori et al.* (RB-277462). **Minas Gerais:** "in locis uliginosis Bello Horizonte," A. Silveira, Hb. Silveira 285 (R); prope Barbacena, A. Silveira, Hb. Silveira 285 (R); Ouro Preto, Escola de Farmácia de Ouro Preto, *Fontella 1065 & Badini* (RB); Rancharia, *Valente & Badini* (RB-201604); Mariana, Vazamento de Lixo, *Valente & Badini* (RB-201618). **Rio de Janeiro:** Nova Friburgo, Alto Macaé, *Glaziou 6903a* (R); Petrópolis, Herb. J. Saldanha, Gab. de Bot. da Esc. Politécnica *n. 5245* (R). **São Paulo:** São Paulo, Dom Pedro, Jacarei, Campinas, Atabaia, *Gentry & Zardini 49250* (RB); Iporanga, *Pabst 6759 & Pereira 6933* (B, HB).

15. Oxypetalum pannosum Decne., Prodr. (DC.) 8: 583. 1844. *Gothofreda pannosa* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. São Paulo: *C. B. Gaudichaud 273* (holotype, P!, P photo at RB!).

15a. Oxypetalum pannosum var. **pannosum** Decne.

Twining vine, branches lanose. Leaves with petiole 2–2.2 cm, lanose, with 3 or 4 colleters at blade base; blades 7.5–8.0 × 2.5–2.6 cm, ovate-triangular to oblong-ovate, camptobrochidodromous, basally cordate or ± cordate, apically acuminate, adaxially tomentose, abaxially whitish lanose. Inflorescence 8- to 9-flowered, cymoid to corymbiform; peduncle 5–6 mm, lanose; pedicels 1.8–2 mm, lanose. Floral buds 0.6–0.7 cm, elliptical or conical; calyx lobes 4–5 × 1–2 mm, with 1 colleter below each sinus within, linear-lanceolate, abaxially tomentose, adaxially puberulent, with smooth trichomes 186–352 µm; corolla campanulate, tube 1.8–2 mm, glabrous outside, with smooth trichomes 103–453 µm, puberulent inside, lobes 9.5–10 × 2–2.5 mm, lanceolate, reflexed, twisted, adaxially and abaxially pubescent, with smooth trichomes; corona lobes 1.8–2 × 1.3–1.8 mm, exserted, obovate or ± obovate, apex apically truncate, obscuring the anthers, adaxially with tooth reaching up to middle of the corona; gynostegium 1.8–2 mm high, ± sessile; anthers ± quadrate, with wings 1.4–1.5 mm, slightly shorter than dorsum; connective appendage 1.8–2 mm, sagittate-triangular, higher than corona and corpusculum; corpusculum 0.90–0.99 × 0.26–0.30 mm, in frontal view ossiform, in lateral view thickened, not laminar, curved; caudicles 0.10–0.21 mm, horizontal, shortly stipitate, teeth lateral, curved and free; pollinia 0.90–1.07 × 0.21–0.29 mm, oblong-cylindrical, slightly curvate, equal or approximately equal in size to corpusculum; style-head 3.8–4.8 mm, ± conical at base, bifid at middle, branches filiform and divergent. Follicle ca. 8.5 mm, fusiform, with acuminate tip, woolly whitish (*A. Loefgren 8883*).

Phenology. Collected in flower in January and from October to December; in fruit in December.

Distribution. Brazil: Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo. Argentina; Paraguay; Uruguay.

Oxypetalum pannosum var. *pannosum* is found in low montane and montane rainforest, rainforest with *Araucaria*, and grasslands from 450 to 1000 m in Brazil. This species was found for the first time in Rio de Janeiro and is recognized by its leaf blades abaxially whitish lanose and its corpusculum ossiform with a split broader above the middle. It is similar to *O. banksii* subsp. *banksii*, *O. arachnoideum*, *O. cordifolium* subsp. *brasiliense*, *O. cordifolium* subsp. *pedicellatum*, *O. pilosum*, and *O. wightianum* by the corpusculum equal, approximately equal, or slightly longer than the pollinia and the corolla lobes spreading or reflexed. It differs from these taxa in the ossiform shape of the corpusculum and the corona lobes with tooth reaching to the middle of the lobe (vs. tooth short, absent, or with thickened corolla lobes).

Hoehne (1916) described *Oxypetalum pannosum* var. *macranthum* (as "macrantha"), probably from the collection of Dusen and Malme collected in Curitiba. Examination of *Malme 422* (isosyntype, R) showed that *O. pannosum* var. *macranthum* differs from var. *pannosum* in the shape and size of the corolla lobes, the shape of the corona lobes, and the style-head thick, ± conical at base, abruptly attenuated, and divided in cylindrical branches. The corpusculum in var. *macranthum* is usually surrounded by a spongy, obovate appendage.

Specimens examined. BRAZIL. **Minas Gerais:** Aiuruoca, *Glaziou 16256* (R). **Rio de Janeiro:** Resende, Visconde de Mauá, *Marquete et al. 453* (R, RB). **Rio Grande do Sul:** Alto da Serra, Ricarda, *Pereira 8544* (HB, RB). **Santa Catarina.** Povoado, Anitápolis, *Klein & Bresolin 10600* (RB). **São Paulo:** Bom Sucesso do Itararé, *Torezan et al. 549* (ESA).

16. Oxypetalum patulum E. Fourn., Fl. Bras. 6(4): 278. 1885. *Gothofreda patula* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Minas Gerais: Serra da Ibitipoca, *A. F. C. P. St.-Hilaire 265* (holotype, P!, P photo at RB!). Figure 7.

Oxypetalum hirsutum Silveira, Fl. Serr. Min.: 27. 1908. TYPE: Brazil. Minas Gerais: "in pratis arenosis in Serra do Papagaio, prope Ayuruoca," Nov. 1897. *Silveira 315* (holotype, R!).

Decumbent shrub, branches villose-hirsute. Leaves with petiole 1–5 mm, villose-hirsute, with 2 colleters at blade base; blades 1–2 × 0.6–1.2 cm, ovate or elliptical, brochidodromous, apically acute or mucronate, basally cordate or rotundate, adaxially and abaxially slightly villose. Inflorescence 3- to 4-flowered, sciadioidal; peduncle 1–2 cm, villose; pedicels 6–10 mm, villose. Floral buds 0.8–0.9 cm, conical; calyx lobes 4–6 × 1–1.2 mm, with 4 or 5 colleters below each sinus within, linear, abaxially villose, adaxially pubescent toward apex and glabrous or glabrescent at base, with smooth trichomes 197–954 µm; corolla campanulate, tube 1.8–2 mm, pubescent outside, puberulent inside, lobes 8–9 × 1–2 mm, lanceolate, spreading, twisted, abaxially villose, adaxially glabrous, with smooth trichomes; corona lobes 1.8–2 × 1–1.5 mm, rectangular-spathulate, apex rounded or obtuse, inconspicuous tooth adaxially present at apical portion; gynostegium 1–1.5 mm high, ± sessile; anthers ± rhombic, with wings 0.8–1 mm, longer than dorsum; connective appendage 0.5–0.7 mm; corpusculum 0.42–0.49 × 0.12–0.14 mm, sagittate or ± sagittate, thick, not laminar, planar; caudicles 0.11–0.12 mm, fixed on base of corpusculum, ± horizontal, broad and planar, teeth included; pollinia 0.49–0.52 × 0.14–0.15 mm, elliptical, very slightly longer than corpusculum; style-head 4–5 mm, delicate, elongate and exserted, bifid from the middle with linear branches. Follicle ca. 3.7 × 0.7 cm, immature, lanceolate, with elongate-acuminate tip, villose; seed not seen.

Phenology. Collected in flower in January, February, and November; in fruit in February.

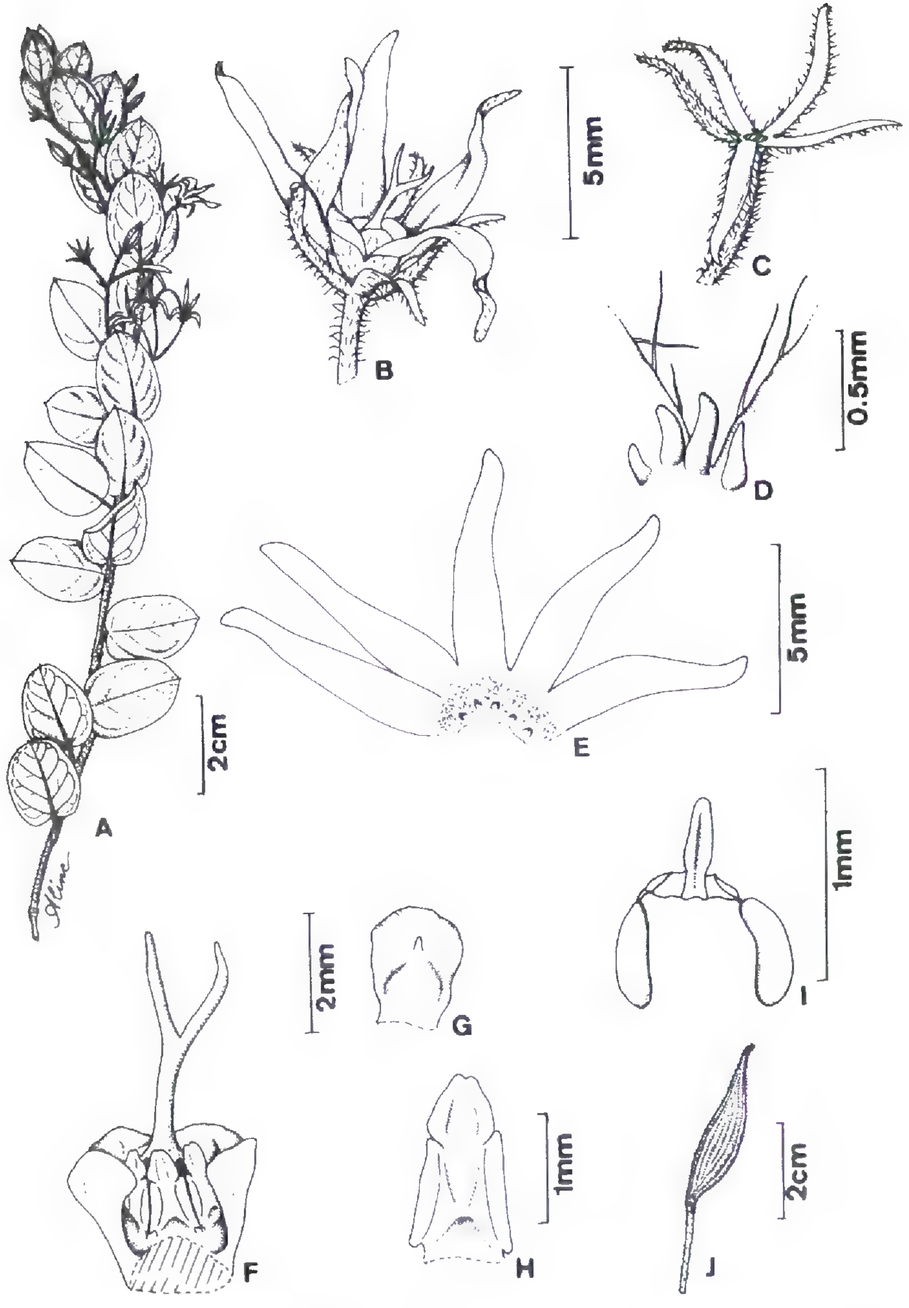
Distribution. Brazil: Minas Gerais and Rio de Janeiro.

In Brazil, *Oxypetalum patulum* is found in high-altitude grasslands and campos rupestres at ca. 2000 m. This species is quite similar to *O. foliosum* Mart. in its slightly villose leaves, the shape of corona lobes, the caudicles with teeth included, and sagittate or ± sagittate corpusculum. It differs from this taxon principally in its corona lobes rounded or obtuse at apex (vs. corona lobes truncate at apex) and horizontal caudicles (vs. descending caudicles).

Specimens examined. BRAZIL. **Minas Gerais:** Lima Duarte, Ibitipoca, Parq. Est. Ibitipoca, Pico do Pião, *Marquete et al. 340a* (RB). **Rio de Janeiro:** Itatiaia, Serra Negra, *Porto 2856* (RB); Parq. Nac. de Itatiaia, Planalto, *Emmerich 605 & Andrade 642* (R); Planalto, *Brade 20198* (RB).

17. Oxypetalum pilosum Gardner, London J. Bot. 1: 539. 1842. TYPE: Brazil. Rio de Janeiro: "among bushes on the ascent of the Pedra Bonita, at Tijuca," *G. Gardner 226* (holotype, K!, K photo at HB!, F!). Figure 8.

Oxypetalum propinquum Decne. in DC. Prodr. 8: 582. 1844. nom. illeg. *Gothofreda propinqua* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Brasilia Prov.: Rio de Janeiro, *Gardner 226* (holotype, P!; isotype, G!).



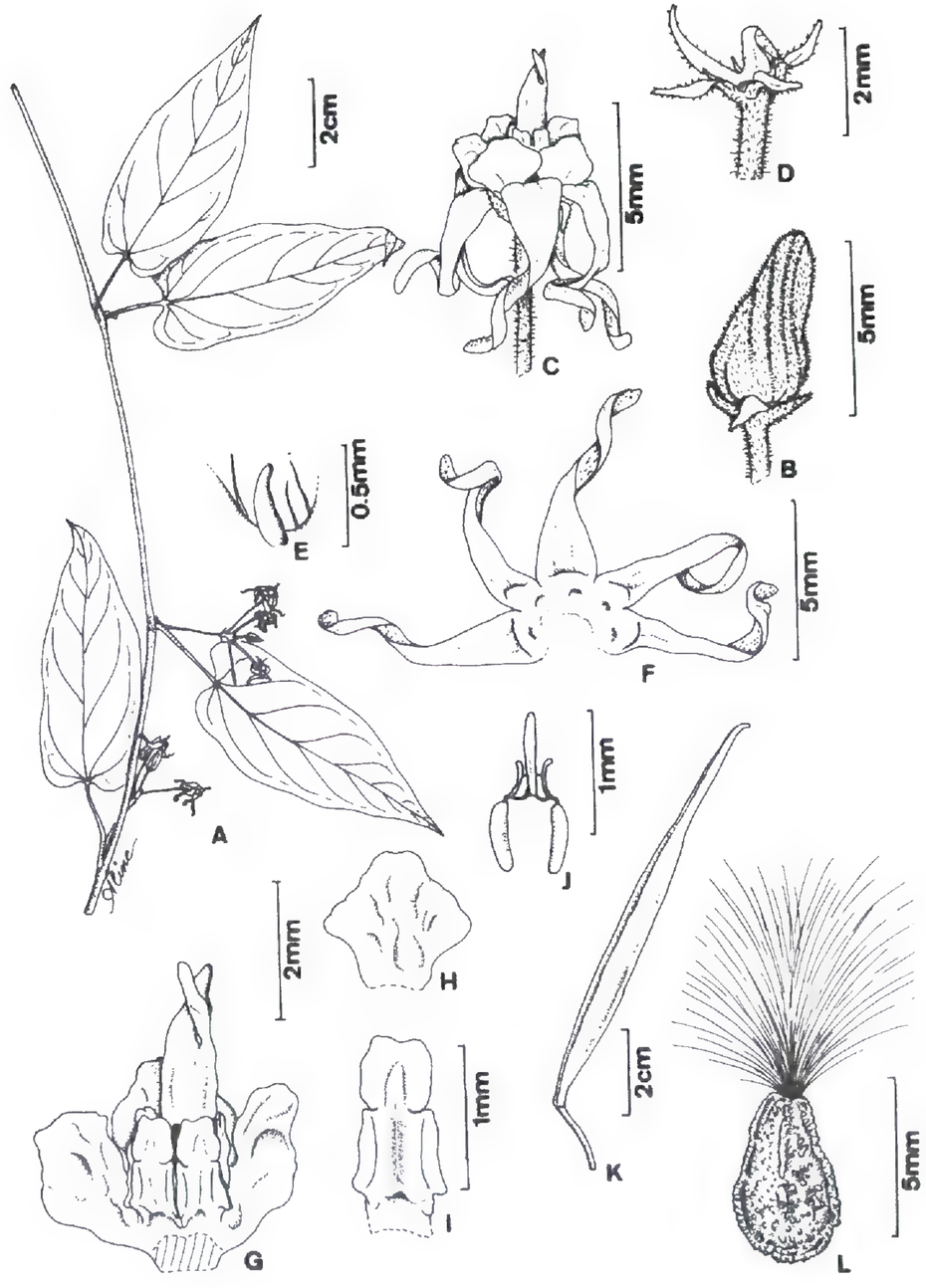


Figure 7 (left). *Oxypetalum patulum*. —A. Flowering branch. —B. Flower. —C. Calyx. —D. Colleters in calyx lobe sinus. —E. Corolla lobes, adaxial view. —F. Corona and gynostegium with corolla lobes removed. —G. Corona lobe, adaxial view. —H. Anther, abaxial view. —I. Pollinarium. —J. Fruit. Drawn from *Brade 20198* by A. S. Lima.

Figure 8 (right). *Oxypetalum pilosum*. —A. Flowering branch. —B. Floral bud. —C. Flower. —D. Calyx. —E. Colleters in calyx lobe sinus. —F. Corolla lobes, adaxial view. —G. Corona and gynostegium with corolla lobes removed. —H. Corona lobe, adaxial view. —I. Anther, abaxial view. —J. Pollinarium. —K. Fruit. —L. Seeds. Drawn from *Sucre 1467* by A. S. Lima.

Oxypetalum deltoideum E. Fourn., Fl. Bras. 6(4): 269. 1885. *Gothofreda deltoidea* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891, as "*deltodea*." TYPE: Brazil. Rio de Janeiro, *Reidel s.n.* (holotype, LE!).

Twining vine, branches pubescent. Leaves with petiole 12–30 mm, tomentose, with 3 colleters at blade base; blades 2.5–5.5 × 1.5–3.3 cm, ovate, ovate-lanceolate, or ± oblong, camptobrochidodromous, basally cordate, apically acuminate, adaxially pubescent, abaxially tomentose to minutely tomentose. Inflorescence 8- to 9-flowered, cymoid to corymbiform; peduncle 1–1.8 cm, tomentose; pedicels 4–10.5 mm, tomentose. Floral buds 0.4–0.6 cm, ovate-triangular or conical; calyx lobes 2–3.5 × 0.5–0.8 mm, with 1 colleter below each sinus within, triangular-lanceolate, abaxially tomentose, adaxially glabrous, with verrucose trichomes 60–124 µm; corolla campanulate, tube 1.5–2 mm, tomentose outside, glabrous inside, lobes 4.5–9 × 0.5–1.5 mm, linear, broad at base, reflexed, spiralled, abaxially tomentose, adaxially glabrous, verrucose trichomes; corona lobes 2.0–2.5 × 1.4–2.3 mm, convex, adaxially tooth absent; gynostegium 4–5 mm high, ± sessile; anthers ± quadrate or ± rhombic, with wings 0.5–0.8 mm, longer than dorsum; connective appendage 0.7–0.9 mm, ovate; corpusculum 0.6–0.7 × 0.08–0.12 mm, oblong, thin, not laminar, planar; caudicles 0.1–0.16 mm, broad, horizontal, shortly stipitate, with free teeth lateral, curved, exserted; pollinia 0.4–0.58 × 0.12–0.14 mm, oblong; style-head 2–4 mm, elongate and exserted, thicker at base, bifid from the middle, with slightly thick, linear branches. Follicle 5–9.8 × 0.5–0.7 mm, fusiform-elongate, with elongate-acuminate tip, pubescent; seeds 6.5–7 × 4–1.5 mm, ovate, slightly convex, coma 1.5–2 mm.

Phenology. Collected in flower from January to May, in July, and from October to December; in fruit from February to May and from October to December.

Distribution. Brazil: Bahia, Minas Gerais, Espírito Santo, and Rio de Janeiro.

Oxypetalum pilosum is found in secondary rainforest, low montane and montane rainforest, and campos rupestres in Brazil from 400 to 1200 m. This species is quite similar to *O. erostre* E. Fourn. in its leaves, corona lobes ± rhombic to rounded at the apex, and the pollinarium. It differs from this species principally in its longer corolla lobes (vs. shorter corolla lobes) and the longer and exserted style-head (vs. shorter and less exserted style-head).

Specimens examined. BRAZIL. **Bahia:** Serra Sincorá, Lagoa Encantada, Ibicoara, Brejão, *Harley et al. 15821* (RB). **Espirito Santo:** Santa Teresa, Mata Fria, Clerio Loss, *Koollmann et al. 1704* (HRCB). **Minas Gerais:** Sabará, *Hoehne s.n.* (S, SP-25096). **Rio de Janeiro:** Nova Friburgo, Morro da Cruz, Colégio Anchieta, *Klein et al. 286* (RB); Petrópolis, Carangola, *Góes & Constantino 885* (RB); Rio de Janeiro, Floresta da Tijuca, Bom Retiro, *Fromm 1143 & Trinta 120* (HB, R); Varre e Sae, *Pereira 26* (HB, RB).

18. Oxypetalum regnellii (Malme) Malme, Ark. Bot. 21A(3): 30. 1927. *Calostigma regnellii* Malme, Kongl. Svenska Vet.-Akad. Handl. 34(7): 66. 1900. TYPE: Brazil. Minas Gerais: Caldas, Pedras Brancas Loco aprico, 28 Dec. 1856, *A. F. Regnell III:73* (lectotype, designated here, S!; isotype, R!).

Twining vine, branches pubescent or slightly pubescent. Leaves with petiole 1–6 cm, pubescent or tomentose, with 4 or 6 colleters; blades 5–16.3 × 2.2–8 cm, ovate-triangular, camptobrochidodromous, apically acute or acuminate, basally cordate or sagittate-cordate, adaxially pubescent or tomentose, abaxially densely pubescent or tomentose. Inflorescence 3- to 4-flowered, cymoid to corymbiform; peduncle 5–40 mm, pubescent; pedicels 10–20 mm, pubescent or tomentose. Floral buds 0.7–1.2 cm, lanceolate-ovate or lanceolate; calyx lobes 4–7 × 0.5–1 mm, with 1 or 2 colleters below each sinus within, linear-triangular, abaxially pubescent, adaxially puberulent, with smooth trichomes 122–241 µm; corolla campanulate, tube 5–6 mm, pubescent outside, glabrous inside, lobes 15–20 × 4–5 mm, triangular-lanceolate, spreading or reflexed, twisted, abaxially pubescent, adaxially glabrous or puberulent, with smooth trichomes 53–89 µm; corona lobes 5–7 × 2–3.5 mm, trullate or ± trullate, apex acuminate, tooth adaxially short at base; gynostegium 2–2.5 mm high, ± sessile; anthers ± rectangular, with wings 3.5–4 mm, longer than dorsum; connective appendage 1.2–1.4 mm, ± triangular; corpusculum 1–1.40 × 0.20–0.25 mm, ± claviform, conspicuously longer than pollinia, thick, not laminar, planar; caudicles 0.27–0.39 mm, horizontal, fixed on base of corpusculum, shortly pedicellate, teeth lateral, free; pollinia 0.60–0.70 × 0.16–0.19 mm, oblong, shorter than corpusculum; style-head 3–5 mm, apex crateriform, irregularly laciniate, with 2 lobes longer. Follicle 7–9.5 × 1.7–1.8 cm, ovate-fusiform, with acuminate tip, minutely pubescent; seeds 5–6 × 2.5–3 mm, ovate, verrucose, coma 2.5–3 cm.

Phenology. Collected in flower from January to April, June to August, and October to December; in fruit in April, October, and December.

Distribution. Brazil: Minas Gerais, Rio de Janeiro, and São Paulo.

Oxypetalum regnellii is found in montane rainforest, high-altitude grasslands, and campos rupestres from 1300 to 2200 m in Brazil. This species is quite similar

to *O. insigne* var. *insigne* in its crateriform style-head. It differs from this taxon in its trullate or ± trullate corona lobes (vs. rectangular or oblong corona lobes) and thickened but not laminar corpusculum (vs. a thinner and laminar corpusculum).

Lectotypification of *Oxypetalum regnellii* is necessary because there is no indication in the protologue as to where a holotype specimen was deposited. Of the three syntype specimens distributed, the specimen *Regnell III:73* (S), is selected as lectotype.

Specimens examined. BRAZIL. **Minas Gerais:** Caldas, Pedra Branca, *Regnell III:73* (S, syntype of *Calostigma regnellii* Malme). **Rio de Janeiro:** Itatiaia, Parq. Nac. Itatiaia, Agulhas Negras, Agulhas Negras and Prateleiras, *I. & G. Gottsberger 14-17471* (UB); Teresópolis, Parq. Nac. Serra dos Órgãos, *Occhioni 1428* (RFA). **São Paulo:** São Bento do Sapucaí, Espinha da Pedra do Baú, *Herbier de Alain Jouy 1242* (HRCB).

19. Oxypetalum schottii E. Fourn., Fl. Bras. 6(4): 273, tab. 79, fig. 1. 1885. *Gothofreda schottii* (E. Fourn.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. Rio de Janeiro: *A. F. M. Glaziou 7760* (lectotype, designated here, P!, P photo at RB!; isotype, R!).

Oxypetalum sobralii Occhioni, Dusenia 4(3–4): 271. 1953. Syn. nov. TYPE: Brazil. Rio de Janeiro: Caetetú, Jun. 1944, *D. Constantino & O. C. Goes 623* (holotype, RB!).

Twining vine, branches lanose-tomentose. Leaves with petiole 0.5–1 cm, tomentose, with 4 colleters at blade base; blades 4–6 × 2–3 cm, elliptical or ovate-lanceolate, camptobrochidodromous, apically acuminate, basally cordate or ± cordate, adaxially pubescent, abaxially tomentose. Inflorescence (4)6- to 8-flowered, cymoid to corymbiform, ± sessile; pedicels 15–30 mm, pubescent. Floral buds 0.8–1.3 cm, lanceolate or triangular; calyx lobes 5–6 × 1–1.5 mm, with 1 or 2 colleters below each sinus within, subulate, abaxially puberulent, adaxially glabrous; corolla campanulate, tube 5–6 mm, puberulent outside, glabrous inside, lobes 10–15 × 3–4 mm, linear-lanceolate, spreading or reflexed, spiralled, abaxially pubescent, adaxially minutely papillose or glabrate; corona lobes 3.5–4 × 1.8–2 mm, ± rectangular, apex emarginate and verrucose, tooth adaxially present with fleshy processes lateral at base; gynostegium 2–2.2 mm high, ± sessile; anthers ± quadrate, with wings 1.5–2 mm, longer than dorsum; connective appendage 1.8–2 mm, oblong; corpusculum 1.6–1.8 × 0.68–0.70 mm, oblong, conspicuously longer than pollinia, thin, laminar, planar; caudicles 0.17–0.22 mm, horizontal, fixed on base of corpusculum, teeth present, lateral, free; pollinia 0.45–0.47 × 0.19–0.23 mm, elliptical, 3 to long 4× shorter than corpusculum; style-head 4.8–5 mm, bifid at middle, branches lanceolate and with toothed margin. Fruit not seen.

Phenology. Collected in flower in April, June, August, and November.

Distribution. Brazil: Rio de Janeiro.

Oxypetalum schottii is endemic to Rio de Janeiro, growing in montane rainforest at ca. 945 m. It is quite similar to *O. lanatum* in the shape of its corolla and corona lobes, the style-head, and the laminar corpusculum. It differs from this species principally in its branches and leaves lanose-tomentose, pubescent, or tomentose (vs. the branches and leaves tawny-tomentose or woolly), the caudicles appressed at the base of the corpusculum, and the pollinia 3 to 4× shorter than the corpusculum (vs. pollinia 2× shorter than the corpusculum).

Lectotypification of *Oxypetalum schottii* is necessary because there is no indication in the protologue as to where a holotype specimen was deposited. Of the two syntype specimens distributed, the specimen *Glaziou 7760* (P) is selected.

Specimens examined. BRAZIL. **Brasília:** absque loco speciali, *Schott 5391* (OXF, W, W photo at RB). **Rio de Janeiro:** Caetitú, *Góes & Constantino 623* (RB).

20. Oxypetalum sublanatum Malme, Ark. Bot. 4(3): 6, pl. 1, fig. 3. 1905. TYPE: Brazil. Serrinha, "in paludosis," 15 Jan. 1904, *P. Dúsen 3481* (lectotype, designated here, S!; isotype, R!).

Twining vine, branches villose, lanose, or pubescent. Leaves with petiole 0.7–1.5 cm, villose or tomentose; blades 3.0–6.5 × 1.2–3.0 cm, oblong or ovate-oblong, basally cordate or ± cordate, apically mucronate or acuminate, adaxially lanose or pubescent, abaxially lanose. Inflorescence 2- to 4-flowered, sciadioidal; peduncle 0.4–1.5 mm, lanose; pedicels 11–12 mm, lanose. Floral buds 0.9–1.3 cm, ovate; calyx lobes 7–8 × 1.5–2 mm high, with 7 or 8 colleters below each sinus within, linear-lanceolate, acute to apex, abaxially villose, adaxially glabrous at base and pubescent toward apex, with smooth trichomes 142–678 µm; corolla campanulate, tube 5.5–6 mm, pubescent or tomentose outside, pubescent inside or with trichomes scattered, villose or barbellate at throat, lobes 10–15 × 4–5 mm, triangular-elongated, reflexed, twisted, apex acute or oblique obtuse, abaxially pubescent or ± tomentose, adaxially densely puberulent, pubescent, or tomentose, with smooth trichomes (114)131–361 µm; corona 7–8.2 × 3.5–4 mm, ± rectangular, ± spathulate or oblong, elongate, surpassing the anthers, deeply emarginate at apex, rugose or verrucose, tooth absent; gynostegium 3.5–4 mm high, stipitate; anthers

± rhombic, with wings 1.8–2 mm, longer than dorsum, slightly divergent at base; connective appendage 2–3 mm, ovate or oblong; corpusculum 2.04–4.10 × 0.56–1.02 mm, rectangular or ± rectangular, apex truncate or rotundate, curved in side view, thin, laminar, enclosed by the connective appendages; caudicles 0.28–0.36 mm, horizontal or ± horizontal, teeth 0.47–0.70 mm, free and curved; pollinia 0.70–0.86 × 0.27–0.42 mm, oblong or ± elliptical, with tip rotundate or obtuse; style-head 1.3–3 mm, conical, usually included by corona and usually ± entire at apex. Follicle 5–6.9 × 1.8–1.9 mm, fusiform, apex elongate-acuminate, base acute or obtuse, villose or tomentose; seeds not seen.

Phenology. Collected in flower in January, November, and December; in fruit in June.

Distribution. Brazil: Minas Gerais, Paraná, Rio de Janeiro, Santa Catarina, and São Paulo; Argentina (Meyer, 1944).

Oxypetalum sublanatum is found in high-altitude grasslands, grasslands, campos rupestres, rainforest with *Araucaria*, gallery forest, and marshes in Brazil from 1200 to 1900 m. It is quite similar to *O. lanatum* in habit, shape, the pubescence of the leaf blade, flowers, corona lobes, and laminar corpusculum. It differs from this species principally in its exserted corona lobes that enclose the style-head, which is usually ± entire (vs. the corona lobes only partially enclosing the style-head).

Specimens examined. BRAZIL. **Minas Gerais:** Passa Quatro, Pico do Muro, *Brade 18978 & Silva Araujo* (RB). **Paraná:** Ponta Grossa, Parq. Est. Vilha Velha, *Occhioni 3472* (RFA). **Rio de Janeiro:** Itatiaia, Nova Estrada, *Brade 17272* (RB); Serra do Itatiaia, Planalto, *Pereira 7619* (HB, RB). **Santa Catarina:** Campo Alegre, Morro Iquererim, *Smith & Klein 8506* (RB). **São Paulo:** Bocaina, *Markgraf & Duarte 10261* (RB).

21. Oxypetalum wightianum Hook. & Arn., J. Bot. 1: 288. 1834. *Gothofreda wightiana* (Hook. & Arn.) Kuntze, Revis. Gen. Pl. 2: 421. 1891. TYPE: Brazil. "hedges near Portalagree, rare," *J. Tweedie 227* (holotype, K, K photo at RB!).

Oxypetalum guilleminianum Decne., Prodr. (DC.) 8: 582. 1844. *Gothofreda guilleminiana* (Decne.) Kuntze, Revis. Gen. Pl. 2: 420. 1891. TYPE: Brazil. "prov. Rio de Janeiro, Serra dos Orgãos," *J. B. A. Guillemin 915* (holotype, P!, P photo at RB!; isotype, P fragment at F!).

Oxypetalum squamulatum E. Fourn., Fl. Bras. 6(4): 270. 1885. TYPE: [Brazil.] "in Brasilia meridionali," *F. Sello 4317* (holotype, B!).

Twining vine, branches tomentose. Leaves with petiole 1.5–4 cm, tomentose, with 4 colleters at blade base; blades 4–9(12.5) × 1.5–4 cm, lanceolate-triangular or oblong-triangular-ovate to ovate-triangular, camptobrochidodromous, basally cordate to sagittate, apically acuminate, adaxially pilose or puberulent, abaxially pubescent. Inflorescence 3- to 4-flowered, cymoid to corymbiform; peduncle 3–7.5 mm, tomentose; pedicels 6.6–20 mm, tomentose. Floral buds 0.5–1.1 cm, linear-lanceolate or lanceolate; calyx lobes 3–3.5 × 0.4–0.5 mm, with 1 to 3 colleters below each sinus within, linear-lanceolate, abaxially pubescent, adaxially puberulent, with smooth trichomes 99–209 µm; corolla campanulate, tube 2–2.5 mm, pubescent outside, puberulent inside, lobes 12–15 × 1.5–2 mm, linear or linear-lanceolate, reflexed, spiralled, abaxially pubescent, adaxially puberulent, smooth trichomes 56–136 µm; corona lobes 1.7–2.5 × 1–1.2 mm, trilobate, process rounded or thickened, heart-shaped adaxially up to middle of corona lobes; gynostegium 1.2–1.5 mm high, sessile or ± sessile; anthers ± quadrate, with wings 0.5–0.8 mm, longer than dorsum; connective appendage 1–1.2 mm, ovate; corpusculum 0.5–0.54 × 0.06–0.08 mm, linear, widened above at middle, boat-shaped in outline, thick, not laminar, curved; caudicles 0.1–0.14 mm, horizontal, fixed on base at middle of corpusculum, broad, teeth lateral, curved, free; pollinia 0.36–0.4 × 0.1–0.12 mm, oblong, curved equal to the corpusculum; style-head 4–6 mm, elongate and exserted, bifid from the middle, rarely at base, branches broad and linear. Follicle 4–7.5(9.5) × 1–1.5(4.5) cm, elongate-lanceolate, tuberculate, tomentose; seeds 7.5–10 × 3.2–4 mm, lanceolate-ovate, slightly verrucose, coma 1.2–2 cm.

Phenology. Collected in flower from January to April, June, July, and October to December; in fruit in January, March, April, and May.

Distribution. Brazil: Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo; Paraguay (Fontella-Pereira et al., 1985).

Oxypetalum wightianum is found in low-montane rainforest, montane rainforest, secondary rainforest, grasslands, and often along watercourses in Brazil from 700 to 1800 m. It is quite similar to *O. pilosum* in the corpusculum equal to or slightly longer than the pollinia and the reflexed corona lobes. It differs from this species principally in its trilobate corona lobes that are adaxially thickened and heart-shaped (vs. ± rhomboid, slightly trilobate corona lobes with tooth absent) and a corpusculum that is linear and wider above the middle, boat-shaped in outline (vs. narrowly oblong, rectangular in outline).

Specimens examined. BRAZIL. **Minas Gerais:** Ouro Preto, Morro da Queimada, *Valente & Badini* (RB-201623);

Serra do Antonio Pereira, *Valente & Badini* (RB-201617). **Paraná:** Vila Velha, *Dusén 222a* (BM); Tijucas do Sul, Represa de Vossoroca, *Kummrow 1031* (MBM, RFA). **Rio de Janeiro:** Nova Friburgo, Pico da Caledônia, *Marquete et al. 428* (RB); Resende, Visconde de Mauá, Alto da Serra Selada, *Marquete et al. 452* (R, RB); Rio de Janeiro, Res. Biol. Jacarepaguá, *Castellanos 23421* (GUA); Teresópolis, Estrada Nova, Soberbo, *Occhioni 1286* (RB). **Rio Grande do Sul:** Silveira Martins, *Malme 678* (BM, S). **Santa Catarina:** Blumenau, *Dobereiner & Tokarnia 436* (RB). **São Paulo:** Jundiaí, Serra do Japi, *Leitão Filho et al. 13093* (SPF).

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PYRROLIZIDINE ALKALOIDS AND PHARMACOPHAGOUS LEPIDOPTERA VISITORS OF *PRESTONIA AMABILIS* (APOCYNACEAE) IN A MONTANE RAINFOREST IN ECUADOR[1]

Gunnar Brehm,[2] *Thomas Hartmann,*[3] *and Keith Willmott*[4]

Abstract

Four species of Ithomiinae butterflies (Lepidoptera: Nymphalidae) were observed in nature taking up pyrrolizidine alkaloids (PAs) from withered flowers of *Prestonia amabilis* J. F. Morales (Apocynaceae, Echiteae) in a montane rainforest in southern Ecuador (ca. 1000 m a.s.l.). Quantitative experiments were subsequently carried out using either withered flowers or crushed roots of *P. amabilis*. Field trials were conducted in November 2000 at six locations in the area of the Reserva Biológica San Francisco (1800–2000 m a.s.l., ca. 15 km from the first site) where *P. amabilis* was not known to occur. A total of 40 specimens of 10 species of the Ithomiinae butterflies and 40 specimens of eight species of Arctiidae moths were quantitatively sampled. While the first group showed a clear preference for baits with withered flowers, the latter preferred the crushed roots. In total, 13 species of Ithomiinae were observed visiting PA sources. Eight of these 13 species have no previous records of pharmacophagy for PAs. Within the Ithomiinae, there is evidence of phylogenetic difference in attraction, with a noticeably high proportion of recorded species belonging to the Napeogenini. Analysis of plant material by gas chromatography–mass spectrometry revealed the presence of two novel retronecine monoesters of the lycopsamine type, tentatively identified as 5′-demethyllycopsamine (ideamine A) and 5′-demethylisolycopsamine or one of its diastereoisomers. The highest PA levels were found in flowers of *P. amabilis* (0.13%, dry weight basis), followed by roots (0.075%) and leaves (0.044%). In flowers, ideamine A accounted for 84% of total PAs, whereas roots contained ideamine A (53%) and 32% of its 3′-acetyl ester. We suggest that *Prestonia* R. Br. may have served as an ancestral source of PAs in the evolution of pharmacophagous behavior in the Ithomiinae.

Key words: Apocynaceae (Echiteae), Arctiidae, chemical defense, Ctenuchini, Euchromiini, Ithomiinae, *Prestonia*, pyrrolizidine alkaloid.

Plants have developed a large array of secondary substances for protection against herbivores, while the herbivores have found various ways to overcome these chemical defenses. One of the most remarkable phenomena that has resulted from this evolutionary arms race is the ability of certain herbivores not only to cope with the plant's secondary defensive compounds, but even to sequester and use these substances for their own benefit (e.g., Nishida, 2002). The pyrrolizidine alkaloids (PAs) are one of the best-studied examples. The ability of insects to sequester PAs is phylogenetically widespread. Examples include chrysomeline beetles (Ehmke et al., 1999; Hartmann et al., 1999; Pasteels et al., 2003), ithomiine and danaine butterflies, and arctiid moths. Insect species obtaining PAs from plants avoid accumulation of the toxic PA free base (Hartmann & Ober, 2000). Adapted Lepidoptera convert the PA free

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base into its nontoxic *N*-oxide (Lindigkeit et al., 1997; Brückmann et al., 2000; Naumann et al., 2002), whereas the *N*-oxide is easily reduced in the gut of unspecialized herbivores and predators. PAs are taken up either with eaten plant material or independently from nutrition, the latter behavior termed pharmacophagy (Boppré, 1984).

This paper examines the pharmacophagous behavior in two Lepidoptera taxa, the Ithomiinae (Nymphalidae) and the Ctenuchini–Euchromiini clade (Arctiidae, Arctiinae), visiting PA-containing parts of *Prestonia* R. Br. (Echiteae, Apocynaceae) in a montane rainforest in southern Ecuador.

PAs have been detected in several species of the related genus *Parsonsia* R. Br. (Edgar, 1984; Trigo et al., 1996b). Other genera of Apocynoideae reputed to contain PAs are *Alafia* Thouars, *Anodendron* A. DC., and *Pentalinon* Voigt (Hartmann & Witte, 1995). Due to lack of voucher specimens, however, the true identity of the plants analyzed is uncertain, diminishing the usefulness of these reports. Until additional tests show that these genera definitely contain PAs, it is more prudent to consider that in Apocynaceae they are restricted to Echiteae. Fifty-five valid species exist within the genus *Prestonia*; they are distributed in the New World between northern Argentina and Mexico (Morales, 2004). So far, PAs have been recorded in three species: *Prestonia acutifolia* (Benth. ex Müll. Arg.) K. Schum., *P. guatemalensis* Woodson, and *P. portobellensis* (Beurl.) Woodson (Edgar, 1984; Trigo & Brown, 1990; Trigo et al., 1996b; Pasteels et al., 2003). In samples of one additional species, *P. coalita* (Vell.) Woodson, no PAs could be detected (Trigo & Brown, 1990), but unconfirmed records suggest that plants in other populations of *P. coalita* contain PAs (Trigo et al., 1996b).

The New World Ithomiinae form a monophyletic clade with the Danainae and the Australasian genus *Tellervo* Kirby (Ehrlich, 1958; Ackery & Vane-Wright, 1984; Harvey, 1991; Brower, 2000; Freitas & Brown, 2004). Within this clade, the PAs are sequestered mostly by male butterflies and used as male pheromone precursors and in defense. Females are thought to obtain PAs with the spermatophore from males during mating (Brown, 1987). PAs provide a chemical defense against spiders such as *Nephila* Leach and other predators (Brown, 1984, 1987; Trigo et al., 1996a; Silva & Trigo, 2002; but see Collins & Watson, 1983; Pinheiro, 1996).

In addition to the ithomiine and danaine butterflies, the globally distributed arctiid moths are the other large group of Lepidoptera that has independently evolved the ability to sequester PAs for use as male pheromone precursors and chemical defense. Many arctiid moths sequester PAs from PA-containing host plants as larvae (Weller et al., 1999), while others, especially in the Ctenuchini and Euchromiini, collect them as adults. PAs are transferred during mating to the females, and it has been shown that eggs of the arctiid moth *Utetheisa ornatrix* L. are efficiently protected against various egg predators and parasitoids (Eisner et al., 2002). Arctiid moths are generalists or host specialists, and they take up PAs either as larvae or as adults (or not at all, or as both; Boppré, 1990). Larval pharmacophagy has been demonstrated, for example, in *Creatonotos* Hübner (Boppré, 1984) and in *Estigmene acrea* Drury (Bernays et al., 2002; Singer et al., 2004), while adult pharmacophagy occurs in many arctiid genera (e.g., Pliske, 1975a; Goss, 1979).

Adult Lepidoptera use two principal natural sources of PAs in the Neotropical region: nectar and withered plant tissue of PA-containing plants. So far, the uptake of PAs with nectar has been observed only from certain plants of the Asteraceae (Eupatorieae) that often attract a specific guild of insects adapted to PAs (Arctiidae: *Senecio* L. (Jörgensen, 1913); Ithomiinae: *Adenostemma* J. R. Forst. & G. Forst., *Ageratum* L., *Emilia* Cass., *Eupatorium* L., and *Trichogonia* (DC.) Gardner (Moss, 1947; Beebe & Kenedy, 1957; Pliske, 1975b; Brown, 1987; Trigo et al., 1996b)). However, the presence of PAs in nectar has not been confirmed in most cases, and it is difficult to distinguish whether flowers are visited for nectar or PAs. PAs may also be obtained from withered or damaged tissue of PA-containing plants, most often on certain plants of the families Asteraceae (*Eupatorium*, *Senecio*), Boraginaceae (*Heliotropium* L., *Tournefortia* L.; Moss, 1947; Beebe, 1955; Pliske, 1975a, b; Brown, 1987), Fabaceae (*Crotalaria* L.), and with unspecified records on Apocynaceae (Brown, 1987). An exceptional case is provided by orchids of the genus *Epidendrum* L.; ithomiine butterflies are attracted by PAs and pollinate the plants, even though nectar is not provided (DeVries & Stiles, 1990).

Material and Methods

Study Area and Field Trials

Our first observations of ithomiine butterflies visiting withered Apocynaceae inflorescences were made on 5 and 12 December 1999 on the forest floor at Río Bombuscaro, near the entrance of Parque Nacional Podocarpus, south of Zamora, Province Zamora-Chinchipe, Ecuador, at ca. 1000 m a.s.l. (4°06.7′S, 78°58.8′W). More observations of ithomiines were made on 4 January 2000 (see Fig. 1), 7 and 27 October 2000, and on 2, 16, and 30 November 2000. *Prestonia* herbaria material (leaves and fresh

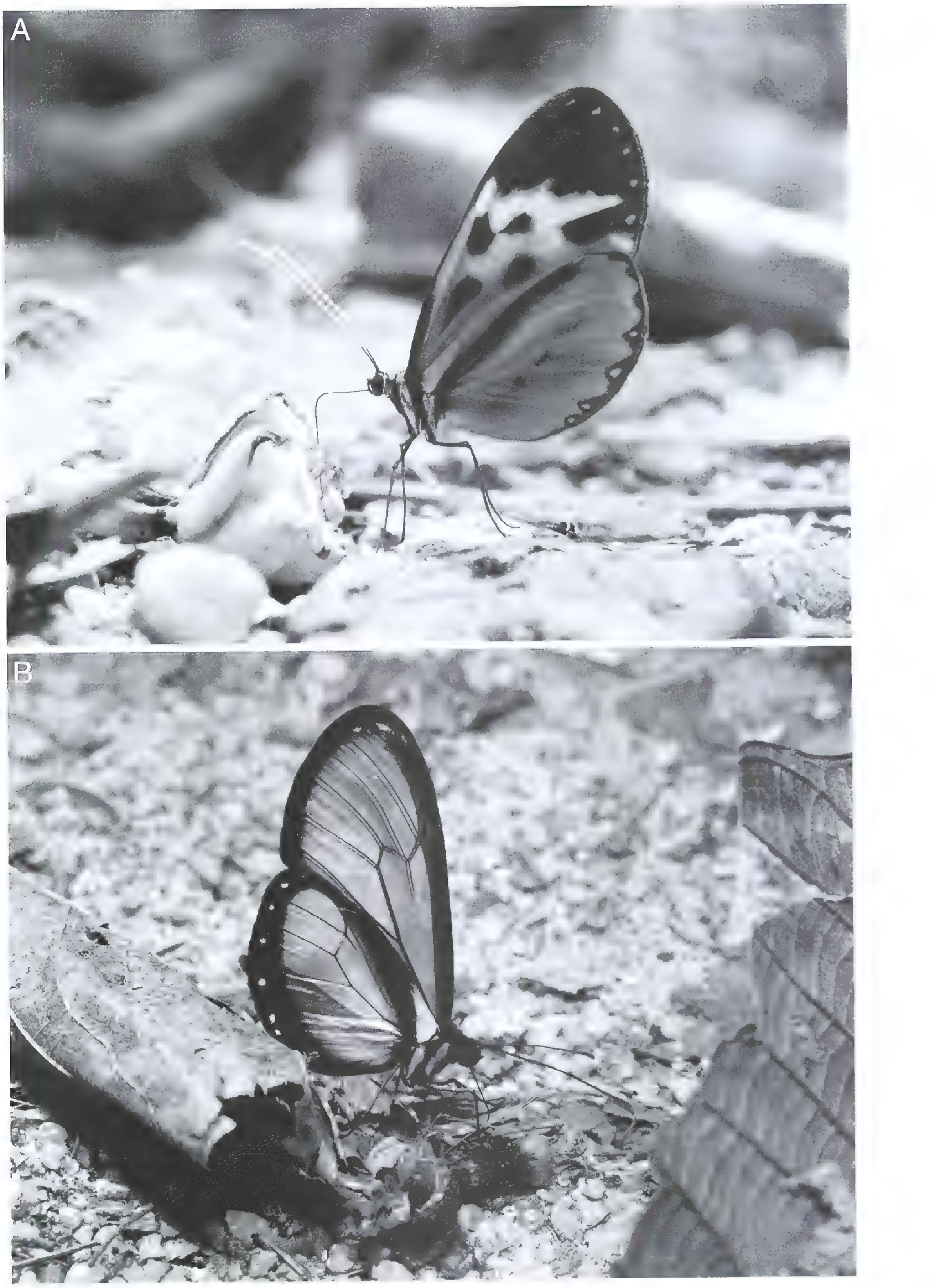


Figure 1. Two common male ithomiine visitors of withered inflorescences of *Prestonia amabilis* as a natural source of pyrrolizidine alkaloids (PAs) in a montane rainforest in southern Ecuador (1000 m a.s.l.), observed on 4 January 2000. Inflorescences fell from the ca. 18 m tall liana to the forest floor. —A. *Hypothyris euclea* subsp. *pyrippe* on a relatively fresh yellow inflorescence. —B. *Hyalyris mestra* subsp. *mestra* on an older blackish inflorescence.

inflorescences of the liana) was collected by F. Werner on 30 November 2000 at a height of ca. 12 m by accessing the canopy using the single-rope technique. The material was sent to a specialist who later described the plant as a new species (Morales, 2004) and deposited the paratype specimen at INBio. Additional material was sampled for baiting experiments: withered inflorescences were collected from

Table 1. PA profiles established by gas chromatography–mass spectrometry for various organs of *Prestonia amabilis* and five visiting Lepidoptera specimens. All PAs represent retronecine esters (see Fig. 2). Itho 3 = *Napeogenes harbona*, undescribed subsp.; Itho 14 = *Hyalyris mestra* subsp. *mestra*; Itho 7 = *Hypothyris euclea* subsp. *pyrippe*; Cte 5 = *Leucopleura* sp. indet., cf. *caucadma*; M+ = molecular mass ion; m/z = mass/charge ratio; PA-X = unidentified pyrrolizidine alkaloid; tr = traces.

			Relative abundance (%)							
			Prestonia amabilis			5 Visiting Lepidoptera specimens				
PAs recovered	m/z [M+]	RI (DB-1 15 m)	Roots	Leaves	Flowers	Itho 3	Itho 14	Itho 7	Cte 5	Cte 5
Retronecine	155	1422	8	15	14	tr	7	25	tr	100
PA-X	?	1655	3	3	2					
9-Angeloylretronecine	237	1801		12						
Supinine	283	1965					2			
Isoideamine A[1]	285	2048	4					20		
Ideamine A[1, 2]	285	2068	53	70	84	tr		55		
Indicine	299	2118				tr	58		tr	
Intermedine	299	2128				tr	21		tr	
Lycopsamine	299	2149					11			
3′-Acetylideamine[3]	327	2185	17							
3′-Acetylideamine[3]	327	2190	15							
3′-Acetylintermedine	341	2235					1			
Total alkaloids (µg/individual):						< 0.8	114	37	< 0.8	2.5
Total alkaloids (mg/g dry wt.):			0.75	0.44	1.31	< 0.1	6.3	6.7	< 0.1	0.4

[1] Tentatively identified; the precise assignment of possible diastereoisomers (see Fig. 2) was not possible.
[2] Occurrence as double peak indicating the presence of stereoisomers (identical mass fragmentation).
[3] Representing two diastereoisomers (identical mass fragmentation).

the ground, and roots were dug up from the soil. Inflorescences and roots were subsequently dried in an oven at ca. 50°C. Roots were crushed in order to increase the release of secondary substances.

Field experiments were carried out between 29 October and 23 November 2000 at six locations in the area of the Reserva Biológica San Francisco (1800–2000 m a.s.l., 3°58.3′S, 79°04.7′W) (see Brehm et al., 2005 for a map) where *Prestonia amabilis* J. F. Morales is not known to occur. There was no precipitation in the period between 31 October and 15 November (the veranillo season), and there was moderate precipitation during the rest of November (G. Brehm, pers. obs.). At each location, two traps were installed close together (10–15 m), ca. 1 m above the ground. Depending upon the availability of plant material, between three and six pairs of traps were operated in parallel. The traps were designed according to DeVries (1987); they were 70 cm high and 40 cm in diameter. Approximately 1 g of PA-containing plant material, consisting of inflorescences or crushed root material, was used as bait in a plastic dish centered in the bottom of the trap. All traps were checked daily between 9:00 and 11:00 a.m., and fresh material (sampled 27 October, 2 and 16 November 2000) was provided every four to five days (inflorescences/root samples were regularly replaced by each other).

Arctiid moths were identified by comparison with type material in the Natural History Museum (London) by G. Brehm, and Ithomiinae butterflies were identified by K. Willmott. Lepidoptera voucher specimens are deposited in the Museum für Naturkunde, Stuttgart, Germany (SMNS). Statistical analyses were performed with the software Statistica 5.5 (StatSoft, Tulsa, Oklahoma).

PYRROLIZIDINE ALKALOID ANALYSIS

Plant materials included air-dried roots (1 g), leaves (0.6 g), and flowers (0.6 g) of *Prestonia amabilis*; these were available for PA identification and quantitative analysis. PAs were investigated in five Lepidoptera specimens (Table 1). Insects were freshly frozen and later spread and dried. It cannot be excluded that PAs might have been partly degraded during relaxation of the insects. Partially oxidized necine bases, generally a good indicator for postmortem degradations, could not be detected in any sample. The preparation of PA extracts from plant and insect samples, their subsequent separation and quantification by capillary gas chromatography, and their identification by combined gas chromatography–mass spectrometry was performed as described previously (Witte et al., 1993; Hartmann et al., 2005). The retention indices (RI) given in Table 1 refer to a 15 m DB-1 capillary column (J&W Scientific Inc., Folsom, California).

Results

Observations in Nature

A total of four species of Ithomiinae were observed at withered inflorescences of *Prestonia amabilis* in the rainforest at 1000 m a.s.l. (Table 2). Figure 1 shows the two most common of these species (*Hypothyris euclea* subsp. *pyrippe* and *Hyalyris mestra* subsp. *mestra*) while taking up PAs from the withered plants through the proboscis. The inflorescences had a sweetish smell. Many butterflies (ca. 50) were observed on 5 and 12 December 1999 when ca. 100 withered inflorescences were present on the ground. Fewer butterfly individuals (ca. 2–20) were observed during subsequent visits to the site (January 2000 and October–November 2000), although many withered inflorescences were available (especially in November 2000). Ithomiine butterflies were also observed in the canopy overhead, but it is unknown whether they were actually visiting *P. amabilis*. A 20-cm-long section of *P. amabilis* was uprooted at 10:00 a.m. on 7 October 2000 and exposed. Six hours later, two males of *H. euclea* subsp. *pyrippe* were observed on this root. Only a single arctiid moth (*Pseudosphex* Hübner) was observed, during the day on 12 December 1999, sitting on withered inflorescences (D. Bartsch, pers. comm.). No other observations of arctiids (by day or at night) were made. However, two species, *Chetone histrio* Boisduval and *Sagaropsis* Hering, were found in the vegetation on 16 November 2000 within a 10-m radius, but it remains speculative whether or not they had visited *P. amabilis*.

Field Trials

The overall capture success of the baited traps was considered moderate. A total of 40 Ithomiinae (10 species) and 40 Arctiidae (8 species) were trapped (Table 2). Several moths and two butterflies escaped while the baits were emptied. By far, the most specimens and species were trapped at one site in a single pair of traps. Collecting data for each trap are available from the first author upon request. The highest numbers recorded during a single day were: Ithomiinae: 3 and 4 individuals (trap 9, 7 November; trap 3, 31 October); Arctiidae: 5 individuals (trap 1, 29 October; trap 2, 10 November). Because none, or only one or two individuals were trapped on most days, the results were summarized for the entire collecting period (Table 2). When all traps and species within the two clades are summarized, it becomes evident that the taxa show different preferences for either withered inflorescences (Ithomiinae) or crushed roots (Ctenuchini–Euchromiini; Table 2). This is confirmed by χ^2 tests of pooled samples (Ithomiinae: $\chi^2 = 16.9$, $P < 0.001$; Ctenuchini–Euchromiini: $\chi^2 = 6.4$, $P < 0.05$, both $df = 1$). Because few individuals were collected in each species, the data do not allow a statistical analysis at species level.

Adult pharmacophagy has previously been observed in only five of the 13 Ithomiinae species recorded in the present study (Table 2). However, pharmacophagy had been reported in other studies for six out of seven genera. The only exception is the recently described genus *Megoleria* Constantino, containing two species, which is sister to *Hyposcada* Godman & Salvin (Willmott & Freitas, 2006). Uniquely within the Ithomiinae, *Megoleria* and *Hyposcada* larvae feed on Gesneriaceae (Drummond & Brown, 1987; A. V. L. Freitas & G. W. Beccaloni, pers. comm.; K. Willmott, pers. obs.), which are not known to contain PAs. In the Arctiidae, pharmacophagy has previously been reported in only one of the eight species listed (*Argyroeides augiades* Druce; Table 2). So far, adult pharmacophagy has been recorded in three of the genera, but it has not previously been reported in three other genera (Table 2).

Other Observations

The species of Ithomiinae and Arctiidae listed in Tables 2 and 3 represent only a part of the local faunas of these taxa. Table 3 provides a list of 15 additional ithomiine species that were collected using an insect net in the study area in October and November 2000, while an additional 23 species have been recorded in this elevational range from the Zamora valley and could be present in the study area (K. Willmott and J. W. Hall, unpublished data). A list of 297 arctiid species recorded for the study area (1800–2005 m a.s.l.) is provided by Hilt and Fiedler (2006).

Pyrrolizidine alkaloids of Prestonia amabilis *and selected insect visitors.* Gas chromatography–mass spectrometry analysis of the plant samples revealed the presence of two novel PAs that were tentatively identified as 5′-demethyllycopsamine (ideamine A) and 5′-demethylisolycopsamine (isoideamine A; Fig. 2).

Ideamine A has not been previously found in plants, but it has been found in a danaine butterfly, *Idea leuconoe* Erichson, feeding on *Parsonsia laevigata* (Moon) Alston (Apocynaceae; Nishida et al., 1991) and in the PA-sequestering Neotropical leaf beetle *Platyphora boucardi* Jacoby, feeding on *Prestonia portobellensis* (Hartmann et al., 2003). In both insects, ideamine A was found to be an insect transformation product of the sequestered macrocyclic

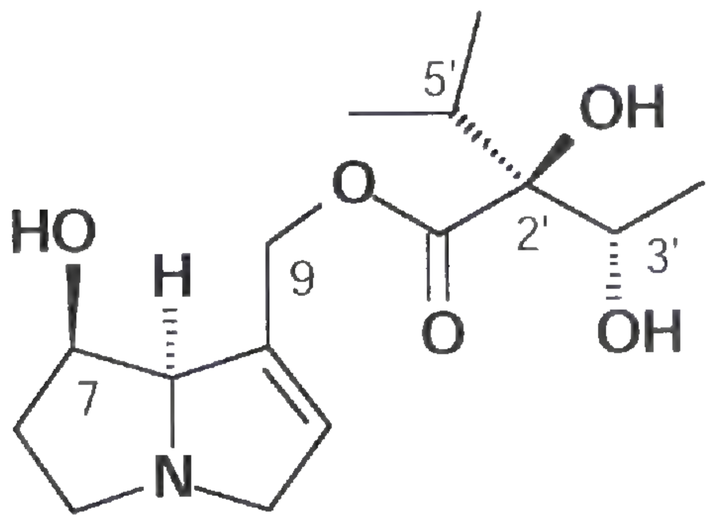
Table 2. Pharmacophagy of 13 Ithomiinae butterflies and eight Arctiidae (Ctenuchini–Euchromiini) moths observed on (1) withered inflorescences and (2) crushed roots of *Prestonia amabilis*. Lepidopterans were attracted using baited traps in a montane rainforest in southern Ecuador (1800–2000 m a.s.l.). All Ithomiinae and most Arctiidae specimens were males. Females were trapped in *Leucopleura* sp. (1 out of 10), *Cyanopepla imperialis* (2 out of 6), and *Argyroeides augiades* (4 out of 5). Pharmacophagy toward PAs has previously been observed for five of the 13 Ithomiinae species listed (Beebe, 1955; Pliske, 1975b; Edgar & Pliske, 1976; Brown, 1985, 1987). PA pharmacophagy has previously been reported for only one of the listed Arctiidae species and has so far not been recorded in the genera *Leucopleura*, *Mevania*, and *Microgiton* (Jörgensen, 1913; Moss, 1947; Beebe & Kenedy, 1957; Pliske, 1975b; Goss, 1979; Häuser & Boppré, 1997). Records identified as "new" are at a global scale. d/n = diurnal/nocturnal behavior was observed (nocturnal observations: Hilt & Fiedler, 2005); qual = qualitative observations of pharmacophagy on withered flowers made at the original collecting site of *P. amabilis* (ca. 1000 m a.s.l.); KW = K. Willmott; MS = manuscript in preparation.

ID	Species	Inflorescence trap no.							Root trap no.							d/n	Pharmacophagy
		1	3	5	7	9	11	**sum**	2	4	6	8	10	12	**sum**		
	Ithomiinae																
8	*Greta alphesiboea* Hewitson, 1869	-	-	-	-	-	-	**0**	-	-	-	-	1	-	**1**	d	New record for species
4	*Greta andromica* subsp. *andania* Hopffer, 1874	-	-	-	-	1	-	**1**	-	-	-	-	-	-	**0**	d	KW, pers. obs., Ecuador
10	*Greta ortygia* subsp. *ortygia* Weymer, 1890	-	-	-	-	-	1	**1**	-	-	-	-	2	-	**2**	d	KW, pers. obs., Ecuador
1	*Hyalyris antea* subsp. *amarilla* Vitale & Bollino, 2000	3	1	-	-	-	-	**4**	-	-	-	-	-	-	**0**	d	New record for species
2	*Hyalyris praxilla*, undescribed subsp. Willmott & Lamas	5	1	-	-	-	-	**6**	-	-	-	-	-	-	**0**	d	New record for species
14	*Hyalyris mestra* subsp. *mestra* Hopffer, 1874							**qual**								d	New record for species
7	*Hypothyris euclea* subsp. *pyrippe* Hopffer, 1874	-	-	-	-	-	1	**qual**	-	-	-	-	-	-	**0**	d	Known from other subsp.
12	*Hypothyris mansuetus* subsp. *amica* Weymer, 1883							**qual**								d	New record for species
11	*Ithomia agnosia* subsp. *agnosia* Hewitson, 1855							**qual**								d	KW, pers. obs., Ecuador
6	*Megoleria orestilla* subsp. *orestilla* Hewitson, 1867	-	-	-	-	-	-	**0**	-	-	1	-	1	-	**2**	d	New record for genus
3	*Napeogenes harbona*, undescribed subsp. Vitale & Willmott	4	1	1	-	2	-	**8**	1	-	-	-	1	-	**2**	d	New record for species
9	*Napeogenes lycora*, undescribed subsp. Vitale & Willmott, MS	1	-	-	1	-	-	**2**	-	-	-	-	-	-	**0**	d	New record for species
5	*Oleria athalina* subsp. *banjana* Haensch, 1903	3	-	1	-	4	2	**10**	-	-	-	-	-	-	**0**	d	KW, pers. obs., Ecuador
	Sum	16	3	2	1	7	4	**33**	1	-	1	-	5	-	**7**	d	New record for species
	Arctiidae																
3	*Argyroeides augiades* Druce, 1896	2	-	-	-	-	-	**2**	3	-	-	-	-	-	**3**	d/n	Known from species
6	*Cyanopepla argyrtidia* Hampson, 1898	-	-	-	-	-	-	**0**	3	-	-	-	-	-	**3**	d	New record for species; known from genus
1	*Cyanopepla imperialis* Druce, 1883	1	-	-	-	1	-	**2**	3	-	-	-	1	-	**4**	d	New record for species; known from genus
7	*Euagra chica* Hampson, 1898	1	-	-	-	-	1	**2**	2	-	-	1	-	-	**3**	d/n	New record for species; known from genus
5	*Leucopleura* sp. indet., cf. *caucadma* Druce, 1894	3	-	-	-	-	1	**4**	4	1	-	-	1	-	**6**	d/n	New record for genus
2	*Mevania basalis* (?) Walker, 1864	-	-	-	-	-	-	**0**	1	-	-	-	-	-	**1**		New record for genus
8	*Microgiton submacula* Walker, 1854	-	-	-	-	-	-	**0**	-	-	-	1	-	-	**1**		New record for genus
4	*Sphaecosoma* sp.	2	-	-	-	-	-	**2**	4	1	-	-	2	-	**7**	d	Known from genus
	Sum	9	-	-	-	1	2	**12**	20	2	-	2	4	-	**28**		

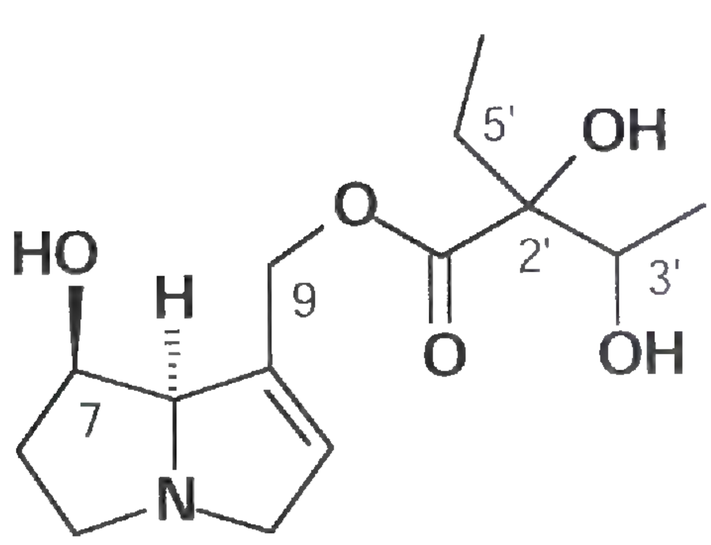
Table 3. Other Ithomiinae species, collected by G. Brehm in the study area in southern Ecuador using an insect net, that were not observed at *Prestonia amabilis* baits. All species that were trapped at *P. amabilis* bait stations (Table 2) were also collected with nets (with the exception of *Megoleria orestilla* subsp. *orestilla*), but are not listed here. Note that butterflies were not sampled quantitatively. However, the numbers suggest a rough estimate of abundance in the area. KW = Keith Willmott; MS = manuscript in preparation.

ID	Species	Individuals collected	Pharmacophagy
10	*Dircenna adina* subsp. *lorica* Weymer, 1875	3	KW, pers. obs., Ecuador
6	*Episcada mira* Hewitson, 1877	2	KW, pers. obs., Ecuador
14	*Godyris dircenna* C. Felder & R. Felder, 1865	1	A. V. L. Freitas, pers. comm.
12	*Godyris hewitsoni*, undescribed subsp. Willmott, MS	2	Records for genus
13	*Greta dercetis* subsp. *dercetis* Doubleday, 1847	1	Record for species (Edgar & Pliske, 1976)
16, 17	*Ithomia avella* subsp. *epona* Hewitson, 1869	1	KW, pers. obs., Ecuador
18	*Ithomia cleora* Hewitson, 1855	2	KW, pers. obs., Ecuador
19	*Ithomia salapia* subsp. *derasa* Hewitson, 1855	2	KW, pers. obs., Ecuador
3	*Ithomia terra* subsp. *terra* Hewitson, 1853	6	KW, pers. obs., Ecuador
9	*Napeogenes flossina*, undescribed subsp. Vitale & Willmott, MS	3	On Asteraceae (KW, pers. obs., Ecuador)
11	*Oleria estella* subsp. *estella* Hewitson, 1868	1	Records for genus
5	*Oleria makrena* subsp. *makrenita* Haensch, 1903	3	KW, pers. obs., Ecuador
20	*Patricia oligyrtis* subsp. *oligyrtis* Hewitson, 1877	4	*Patricia dercyllidas* observed on Asteraceae flowers and tree (KW, pers. obs., Ecuador)
7	*Pteronymia alissa* subsp. *andreas* A. G. Weeks, 1901	6	KW, pers. obs., Ecuador
15	*Pteronymia zerlina* subsp. *machay* T. & L. Racheli, 2003	10	KW, pers. obs., Ecuador

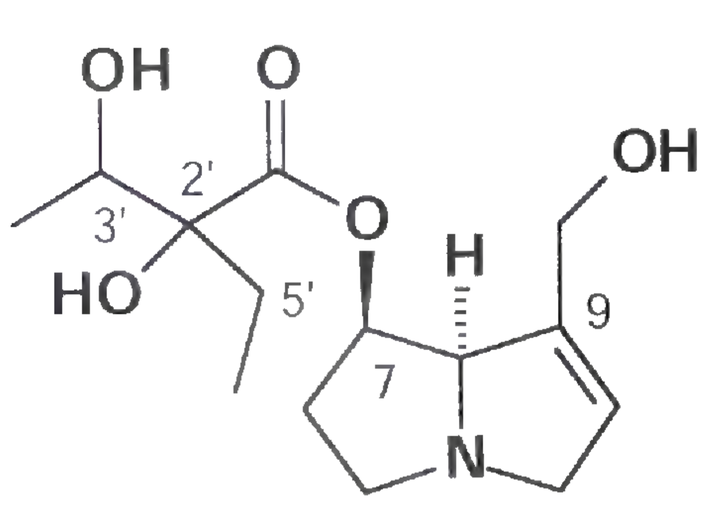
triester alkaloid 14-deoxyparsonsianidine. The stereochemistry of the necic acid moiety at the 2′ and 3′ carbons remains obscure. With all plant samples, the gas chromatography profiles show ideamine A as double peak with identical mass fragmentation patterns indicating the presence of at least two stereoisomers. This is also indicated by the presence of two isomeric 3′-acetyl esters of ideamine A in the



Lycopsamine (7*R*, 2′*S*, 3′*S*)
Intermedine (7*R*, 2′*S*, 3′*R*)
Rinderine (7*S*, 2′*S*, 3′*R*)
Echinatine (7*S*, 2′*S*, 3′*S*)
Indicine (7*R*, 2′*R*, 3′*S*)



5′-Demethyllycopsamine
(ideamine A) or isomer



5′-Demethylisolycopsamine
(isoideamine A) or isomer

Figure 2. Top: Pyrrolizidine alkaloids of the lycopsamine type showing the unique branched-chain C_7 necic acids esterified with retronecine (7*R*) or heliotridine (7*S*). Bottom: The two novel structures identified from *Prestonia amabilis*.

root samples (Table 1). All PAs identified from the plant materials are esters of the necine base retronecine (Table 1).

The two stereoisomers of ideamine A always represent the majority of PAs, ranging from 53% (roots) to 84% of total PAs in flowers (Table 1). Inflorescences display the highest PA concentration (ca. 0.13% alkaloid per dry matter), followed by roots and leaves (Table 1).

Among the five insect samples analyzed, two showed clear plant-derived PA profiles (Table 1). The specimen of *Hypothyris euclea* subsp. *pyrippe* shows the *Prestonia amabilis* pattern, indicating that it obtained its PAs pharmacophagously from this plant species. The specimen of *Hyalyris mestra* subsp. *mestra* did not show the *P. amabilis* PA pattern, but instead contained lycopsamine and two of its isomers (Fig. 2), indicating that it previously gained PAs from the Boraginaceae (e.g., *Heliotropium*) or an Asteraceae (Eupatorieae). The other three specimens, belonging to an undescribed subspecies of *Napeogenes harbona* and to *Leucopleura*, showed only trace amounts of PAs, without a profile indicative for a specific PA-containing host plant.

Discussion

Most ithomiine butterflies are apparently adapted to PAs of the lycopsamine type. These PAs are characterized by their unique branched-chain C_7 necic acid moiety (Fig. 2; Hartmann & Ober, 2000; Weber et al., 1999). The occurrence of PAs of the lycopsamine type is restricted to certain species of three plant families: the Boraginaceae, the Asteraceae (only Eupatorieae), and the Apocynaceae (Hartmann & Witte, 1995). PAs of the lycopsamine type occur mostly as monoesters (Fig. 2), but occasionally also occur as open-chain diesters or macrocyclic triesters. The latter are only known from certain species of the Apocynaceae (e.g., *Parsonsia*). There are several lines of evidence indicating a specific preference of ithomiine butterflies for PAs of the lycopsamine type: (1) PAs recovered from field-caught butterflies are almost exclusively monoesters of the lycopsamine type (Brown, 1984, 1987; Trigo et al., 1996a); (2) the butterflies specifically transform the various diastereoisomers (see Fig. 2) into lycopsamine and intermedine, which represent the major PAs recovered from ithomiines (Trigo et al., 1994, 1996a); (3) feeding experiments revealed that other monoesters (e.g., senecioylretronecine) and macrocyclic PAs of the senecionine type are less efficiently sequestered and are not stably retained (Brückmann et al., 2000); (4) most species convert the specific C_7 necic acid of lycopsamine PAs into male pheromones (i.e., ithomiine γ-lactones), which are released from the male hairpencils (Schulz, 1998; Schulz et al., 2004). In this context, it is remarkable that *Prestonia amabilis* appears to be attractive for ithomiines, although it contains the two novel PAs, ideamine A and isoideamine A, which, instead of the unique C_7 necic acid, possess the respective lower homologues with one methyl group less (Fig. 2). Because neither lycopsamine nor one of its diastereoisomers could be detected in *P. amabilis*, ideamine A must be responsible for the attractiveness of the plant to ithomiines.

Withered inflorescences of *Prestonia amabilis* are apparently a natural source of PAs for Ithomiinae and Arctiidae in our study area, although only relatively few species (four of Ithomiinae and one of Arctiidae) were actually observed feeding in a natural situation. Our study demonstrates for the first time unambiguous pharmacophagy by Lepidoptera on *Prestonia*. The only previous hint was provided by Trigo et al. (1996b), who reported visits of two males and one female of the primitive ithomiine *Tithorea harmonia* on flowers of *Prestonia acutifolia*. The fact that pharmacophagy was observed for the first time for one ithomiine and three arctiid genera, as well as for most species (8/13 Ithomiinae species, 7/8 arctiid species), suggests that many more such relationships are likely to exist in Neotropical rainforests (see also Häuser & Boppré, 1997).

Observations of unmanipulated pharmacophagous behavior are generally scarce in the Neotropical region. Pharmacophagy has hardly been reported from regions such as the Andean montane forests in Peru and Ecuador, where the greatest diversity of Ithomiinae and Arctiidae occurs. Most previous observations have been made with uprooted plants, especially by using the highly attractive *Heliotropium indicum* L., other species of *Heliotropium*, and *Eupatorium* species (Moss, 1947; Beebe, 1955; Beebe & Kenedy, 1957; Pliske, 1975a; Goss, 1979; Häuser & Boppré, 1997). These experimental manipulations give important insights into the behavior of the insects. However, little is still known about the butterflies' and moths' preferences for different PA resources in their habitats. Brown (1987) provided a list of Ithomiinae genera that visited either withered Boraginaceae plants or withered plants plus nectar of Eupatorieae (Asteraceae). Species within most Ithomiinae genera were strongly attracted to both sources, but females were obviously less attracted to decomposing plants and more often attracted to the Eupatoriae flowers. This finding is supported by our study, in which exclusively males of Ithomiinae were collected at the *Prestonia* PA sources (that provide no nectar). However, Brown (1987) also showed that not all

Table 4. Comparison of potential and actual numbers of Ithomiinae recorded in pharmacophagy in the study area in southern Ecuador. Potential numbers are taxa recorded from Río Bombuscaro and in the province of Zamora-Chinchipe between 1800–2100 m by K. Willmott and J. W. Hall (unpublished data) during four months of field work over a 10-year period (1993–2003).

Tribe	Potential species	Recorded pharmacophagous species
New tribe (*Athesis–Patricia*)	2	0
Dircennini	19	0
Godyridini	15	3
Ithomiini	5	1
Mechanitini	1	0
Melinaeini	1	0
Napeogenini	10	7
Oleriini	10	2
Total	63	13

genera were equally attracted; a weak response was recorded in some notably primitive (Willmott & Freitas, 2006) genera (*Tithorea* and *Methona*), perhaps because they already sequester PAs from their host plants.

The lists provided in Tables 2 and 3 represent incomplete samples of pharmacophagous Ithomiinae and Arctiidae of the study area; a longer sampling period and broader elevational range would result in additional records of rare species. Nevertheless, some phylogenetic patterns are apparent. For example, *Ithomia terra* subsp. *terra*, *Pteronymia alissa* subsp. *andreas*, and *P. zerlina* subsp. *machay* were all relatively abundant, with six individuals of each species recorded, but none of these species were recorded at baits. Notably, none of the 19 species of ithomiine tribe Dircennini potentially present at the study sites was observed in pharmacophagy, and only three of the 15 species of Godyridini (Table 4), of which *Greta andromica* and *G. ortygia* are two of the most abundant montane ithomiine species, were observed in pharmacophagy. Males of Dircennini and Godyrini are, however, frequently observed feeding at Asteraceae flowers (e.g., Table 3), and this sex-specific behavior strongly suggests they are seeking PAs. It may also be significant that, although the Napeogenini comprise only 10 of the 63 potential species in the study area (16%), seven of the 13 species observed in pharmacophagy are Napeogenini, suggesting a particularly strong attraction within this group of species to *Prestonia*. Napeogenini are characteristic of deep shade, where *Prestonia* occurs, whereas Dircennini and Godyridini are often observed in the forest canopy and at forest edges (K. Willmott & G. Brehm, pers. obs.), where Asteraceae PA-containing flowers grow; this suggests the habitat differences may play a role in preferred PA sources. Elsewhere in Ecuador, we also observed males of three ithomiine species, *Patricia dercyllidas* subsp. *dercyllidas* (undescribed tribe), *Napeogenes larilla* (undescribed subspecies), and *N. flossina* (undescribed subspecies; Napeogenini), feeding on stems and leaves of an apparently undamaged, unidentified Asteraceae tree in cloud forest at 2400 m a.s.l. (K. Willmott, pers. obs.). Deeper forest ithomiines may therefore use a variety of PA sources (e.g., *Prestonia*) about which we know comparatively little.

Among the arctiids, only eight species were recorded at baits. This is a small proportion compared with the many Arctiidae species collected at light traps in the study area. Between 1800 and 2005 m, a total of 297 nocturnal Arctiidae species were recorded (among them 82 Ctenuchini–Euchromiini species; Hilt & Fiedler, 2006). However, only three of the species collected in the *Prestonia* traps were also recorded in these nightly samples (Table 2). Six species found in the traps were also observed flying by day. Previous studies (hand-sampling) recorded a higher number of nocturnal arctiid moths at PA baits than observed in our study (e.g., Beebe, 1955; Häuser & Boppré, 1997). A modification of the trap design (e.g., toward a funnel-design) could possibly increase the number of collected individuals.

Prestonia may have been overlooked previously as a natural source of PA for adult Lepidoptera because the genus consists of vines and lianas. It is more difficult to observe Lepidoptera behavior at plants with this habit than at herbs such as *Heliotropium* or *Eupatorium*. PA-containing Apocynaceae host plants are a key for the understanding of the evolution of PA pharmacophagy in the closely related Danainae, Ithomiinae, and *Tellervo* (Edgar, 1984; Trigo et al., 1996b). Although caterpillars of ca. 95% of ithomiine species feed only on Solanaceae, the likely ancestral host plant family is Apocynaceae, which is found as a host in *Tellervo* and which is a widespread host in the Danainae. Caterpillars of the primitive ithomiine genera *Tithorea*, *Elzunia*, and *Aeria* also use *Prestonia* species as host plants (Brown, 1987; DeVries, 1987; Drummond & Brown, 1987; Trigo & Brown, 1990). Virtually all of the more derived ithomiine species have the ability to sequester PAs and take them up as adults from various plant sources (Brown, 1987; Schulz et al., 2004; Trigo et al., 1996b). It seems likely that a direct ancestor of *Prestonia* may have served as the original additional source of alkaloids for the adults of these species.

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A NEW EXPLANATION FOR THE EVOLUTION OF POLLINIA AND LOSS OF CARPEL FUSION IN *ASCLEPIAS* AND THE APOCYNACEAE S.L.[1]

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ABSTRACT

The demonstration of postzygotic self-incompatibility in *Asclepias* L. prompted us to reconsider explanations for several unusual features of the Apocynaceae. With such late-acting self-incompatibility, mixed loads of self- and cross-pollen can be extremely wasteful by causing abortion of whole fruits and consequent wastage of both cross-pollen and cross-fertilized ovules. We contend that the evolution of pollinia and loss of carpel fusion in certain Apocynaceae represent adaptations to prevent or compensate for these negative effects of mixed pollen loads. Trends in the agglutination of pollen can be seen in the tetrads of *Apocynum* L., which are transported on sticky band-like translators; the masses of tetrads of Periplocoideae, which are deposited onto the sticky scoop portion of a spoon-like translator; the pollinia of Secamonoideae, which have a clip-like translator but no outer covering; and the pollinia of Asclepiadoideae, which are completely enclosed by a waxy outer covering. In many genera of the Rauvolfioideae, the ovary is syncarpous, but in most Apocynoideae and all Periplocoideae and Secamonoideae, the gynoecium consists of two carpels that are free in the ovule-bearing region but fused in the upper region to produce a compitum, which enables pollen placed on one stigmatic area to supply pollen tubes to both ovaries. In at least some Asclepiadoideae, however, a compitum is lacking, although postgenital fusion between the carpel apices still takes place. Parallel trends in pollen delivery and receipt also occur in taxa from other angiosperm families in which late-acting self-incompatibility systems have been implicated.

Key words: Apocynaceae, Apocynoideae, Asclepiadoideae, *Asclepias*, carpel fusion, compitum, late-acting self-incompatibility, Periplocoideae, pollinia, Secamonoideae.

In a letter to Asa Gray in 1857, Charles Darwin wrote:

> "The facts which kept me longest scientifically orthodox are those of adaptation—the pollen-masses in *Asclepias*... To talk of climate or Lamarckian habit producing such adaptation to other organic beings is futile. This difficulty, I believe I have surmounted." (Burkhardt & Smith, 1990: 445)

Given the state of knowledge of genetics in 1857, Darwin certainly can be forgiven for his inability to explain the evolution of milkweed pollinia. Moreover, it is our contention that the explanation lies not in the arena of pollinator–plant coevolution, as Darwin imagined, but rather in the genetics of self-incompatibility. Our work has shown that poke milkweed (*Asclepias exaltata* L.) possesses a postzygotic self-incompatibility system (Lipow & Wyatt, 2000). Such late-acting self-incompatibility is extremely inefficient, often resulting in wasted cross-pollen and cross-fertilized ovules when mixed loads of self- and cross-pollen reach stigmas. This has led to selection for traits that decrease the likelihood of this costly mixing occurring.

These traits include androecial and gynoecial features that affect both pollen delivery and receipt. In the subfamilies Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae (hereafter, APSA clade), there are clear trends toward agglutination of pollen, culminating in complex pollinia, specialized structures that transport large numbers of pollen grains as discrete units, such that the likelihood of mixing of pollen from different donors is minimized. Pollinia are rare, occurring among dicots only in certain derived Apocynaceae, although some other families produce pollen grains that are transported in multiples. Among monocots, analogous (but certainly not homologous) pollinia occur in the Orchidaceae. In the Apocynaceae, there are also evolutionary trends toward increased separation of the

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ovaries and the gynoecial transmitting tissue leading to secondary apocarpy, a trend exactly opposite that found in nearly all other groups of angiosperms (Endress, 1982, 1990). In many Rauvolfioideae, the ovary is syncarpous, but in most Apocynoideae and all Periplocoideae and Secamonoideae, the gynoecium consists of two carpels that are free below and united above in the stylar region. In *Asclepias* L. of the Asclepiadoideae, these apically fused carpels lack a compitum, a shared transmitting tract that distributes pollen tubes to all carpels regardless of the stigma on which pollen is placed (Sage et al., 1990). Therefore, pollen from a given pollinium can reach only one of the two ovaries, again minimizing the likelihood that an ovary will receive pollen from multiple donors.

Here we present a new explanation for the evolution of pollinia and loss of carpel fusion in milkweeds. We then briefly review recent phylogenetic studies of the Apocynaceae s.l. and relate these to evolutionary trends in pollen agglutination and loss of carpel fusion. We also consider earlier explanations for the evolution of pollinia and secondary apocarpy. Finally, we evaluate parallel evolutionary trends in other families of flowering plants and the ability of our new hypothesis to explain them.

A New Explanation

It seems likely that postzygotic self-incompatibility is widespread in *Asclepias*. At least seven species of *Asclepias* are predominantly self-sterile (reviewed by Wyatt & Broyles, 1994; Wyatt et al., 1996, 1998). Although self-pollen tubes grow normally and effect fertilization, fruits are rarely (or never) produced following self-pollinations (Sparrow & Pearson, 1948; Kephart, 1981; Sage & Williams, 1993; Wyatt et al., 1996). Instead, the selfed zygotes do not undergo mitosis, the endosperm divides only a few times, and the selfed ovules consistently abort. Full-sib diallels performed on *A. exaltata* have demonstrated that self-sterility is due to a single gene, apparently with many alleles segregating in local populations (Lipow & Wyatt, 2000). Crosses are incompatible whenever an allele at the S-locus of the diploid pistil matches an allele of the diploid pollen.

Crossing and microscopic studies have identified species in several other genera within the APSA clade that are also self-sterile but show self-pollen tubes growing normally and entering ovules: *Apocynum cannabinum* L. (Apocynoideae), *Periploca aphylla* Decne. (Periplocoideae), and *Gonolobus suberosus* (L.) R. Br. (Asclepiadoideae) (Lipow, 1998; Lipow & Wyatt, 1998, 1999). The genetics that underlie recognition and rejection of self-pollen in these and other genera of the APSA clade are likely the same as in *A. exaltata*, as self-incompatibility systems are typically conserved among closely related taxa (de Nettancourt, 1977). Certainly, the behavior of all these taxa with respect to experimental crosses and ovule penetration is remarkably consistent.

We are not aware of any studies that have examined in detail the nature of self-incompatibility in the Rauvolfioideae (the basal subfamily of Apocynaceae), especially the possibility that it might be late-acting. Such studies could be revealing, however, because in species from several families of Gentianales, incompatible pollen tubes have been observed to arrest in the ovules (Bawa, 1974, 1979; Bawa et al., 1985). The five families currently recognized in the Gentianales fall into two main clades. One is composed solely of the Rubiaceae, whereas the other includes the remaining four families in the following relationship: Apocynaceae are sister to Gelsemiaceae, which together are sister to Loganiaceae s. str., and all three families together are sister to Gentianaceae (Backlund et al., 2000).

We contend that the evolution of postzygotic self-incompatibility created conditions in which compatible cross-pollen was wasted whenever it occurred in mixture with incompatible pollen, because such mixed pollen loads would likely cause the entire fruit to abort. This is because crosses between plants that share even a single self-incompatibility allele are incompatible (e.g., $S_1S_3 \times S_1S_4$). Such diploid control over the compatibility phenotype of the pollen is surprising. Rejection of pollen occurs after penetration of ovules by pollen tubes, but pollen tubes express the haploid, not diploid, male genotype. Thus, in these crosses, one half of the ovules should receive compatible pollen and one half should receive incompatible pollen. Two explanations can account for the observed failure of any seeds to mature from such crosses: (1) recognition occurs much earlier than rejection (hence, the "decision" to abort the fruit is made before fertilization occurs), or (2) fruit maturation requires that more than one half of the ovules within an ovary receive compatible pollen (which seems to be the case in *Asclepias*, as such crosses invariably abort, with fruit-set occurring only from ovaries with a full complement of compatibly fertilized seeds). In either case, a mixed pollen load containing half compatible and half incompatible pollen is unlikely to mature any seeds. This wastes not only incompatible pollen, but also compatible pollen and fertilized ovules. This maladaptive condition may have been the stimulus for the evolution of pollinia and loss of the compitum.

Evolutionary Innovation and Diversification in Floral Morphology

Within the Apocynaceae, it is possible to discern various levels of evolutionary complexity relating to

the formation of pollinia and loss of carpel fusion, as well as several other unique morphological features (Struwe et al., 1994; Liede, 1996; Sennblad & Bremer, 1996; Endress & Bruyns, 2000; Livshultz et al., 2007). Five major groups are often recognized in the Apocynaceae s.l. and assigned subfamilial rank: the Rauvolfioideae and Apocynoideae of the Apocynaceae s. str. and the Periplocoideae, Secamonoideae, and Asclepiadoideae of the Asclepiadaceae (Rosatti, 1989; Leeuwenberg, 1994; Liede & Albers, 1994). The Rauvolfioideae have often been interpreted as a paraphyletic group, from which the other subfamilies have radiated (Schumann, 1895; Pichon, 1948; Fallen, 1986; Sennblad & Bremer, 1996).

Here we focus on the APSA clade. These four subfamilies form a strongly supported monophyletic group characterized by anthers that are adnate to the style-head forming a gynostegium, dextrorse corolla lobe aestivation, an apocarpous gynoecium, and dehiscent follicles containing small seeds with a coma on the micropylar end (Livshultz et al., 2007). Systematic relationships within this group have been controversial. Historically, the Periplocoideae have usually been classified in the Asclepiadaceae (Brown, 1810; Bentham, 1876; Schumann, 1895), but morphological and phylogenetic studies conducted over the past 10 years have suggested, albeit with poor resolution, that the traditional Asclepiadaceae may be biphyletic, with the Periplocoideae and the Secamonoideae–Asclepiadoideae each having a closer relationship to a different group of taxa within the Apocynoideae than they do to each other (Kunze, 1996; Sennblad & Bremer, 1996; Potgieter & Albert, 2001). The close relationship between Secamonoideae and Asclepiadoideae has long been accepted based on morphology alone (Safwat, 1962; Kunze, 1993; Liede, 1996; Fishbein, 2001), and molecular results have confirmed they are sister groups that together form a monophyletic clade (Sennblad & Bremer, 1996). In the broad-based phylogenetic study by Livshultz et al. (2007), resolution within Apocynoideae is much improved, illuminating relationships within the APSA clade, including strong support that the closest relative of Secamonoideae–Asclepiadoideae is a clade of three closely related genera of Apocynoideae, rather than Periplocoideae, and thus confirming the polyphyly of the traditional Asclepiadaceae, a conclusion reached independently by Lahaye et al. (2007).

Evolution of pollinia. The highly derived pollinia of *Asclepias* represent the extreme of an evolutionary trend in the Apocynaceae toward increased agglutination of pollen (Endress, 1994; Endress & Bruyns, 2000). In most genera of the Rauvolfioideae and Apocynoideae, pollen is released as single grains from anthers with two thecae, each of which contains two pollen sacs. In the Apocynoideae, the anthers become postgenitally united with the style-head in various ways. In all subfamilies, the style-head secretes sticky substances that serve as glue for pollen transport (Endress, 1994). Among the Apocynoideae, *Apocynum* L. (Apocyneae) has pollen that is dispersed as tetrads and simple band-like translators that are formed through desiccation of the style-head secretions; band-like translators also occur in *Forsteronia* G. Mey. (Mesechiteae) (Schick, 1982a; Nilsson et al., 1993; Fig. 1A, B). This aggregated transport and mass transfer may reduce the mixing of self- and cross-pollen on the stigmatic surface.

In the Periplocoideae, we see more dramatic evidence of a pollen delivery mechanism that minimizes pollen mixing. Pollen is deposited onto an elaborate translator, which is typically differentiated into a spoon-like pollen receptacle and a stalk with a sticky base (Schick, 1982b; Kunze, 1993; Venter & Verhoeven, 1997; Fig. 1C, D). During pollination, the sticky base of the stalk becomes attached to an insect. When the insect leaves the flower, it pulls out the spoon of pollen, which subsequently may be brought into contact with the stigmatic surface of the style-head when the insect visits another flower (Schick, 1982b). In *Periploca aphylla*, each spoon contains ca. 125 times more pollen grains than there are ovules within an ovary (Wyatt et al., 2000). Thus, a single pollination could deliver more than enough pollen grains to fertilize all of the ovules within an ovary. Pollen is usually shed in tetrads, but in seven of the 33 currently recognized genera, the tetrads of each pollen sac are loosely coherent into pollinia, which lack a sporopollenin outer wall and are not considered to be homologous to those in the Asclepiadoideae–Secamonoideae lineage (Fishbein, 2001; Ionta & Judd, 2007). The pollinia from two pollen sacs of two adjacent anthers are shed onto the adhesive-lined spoon of the translator. Verhoeven and Venter (2001) concluded that pollinia have evolved independently at least twice, and results of a recent molecular analysis of the subfamily indicate that there were probably three to four independent origins of pollinia in the Periplocoideae alone (Ionta & Judd, 2007).

Simultaneous transport of numerous pollen grains in pollinia also evolved independently in the Secamonoideae–Asclepiadoideae lineage. The pollinia of Secamonoideae are superficially similar to those found in Periplocoideae, in being composed of coherent tetrads and lacking an outer sporopollenin wall. Differences in details of the tetrad wall structure (Verhoeven & Venter, 2001; Verhoeven et al., 2003), however, convinced Ionta and Judd (2007) that the

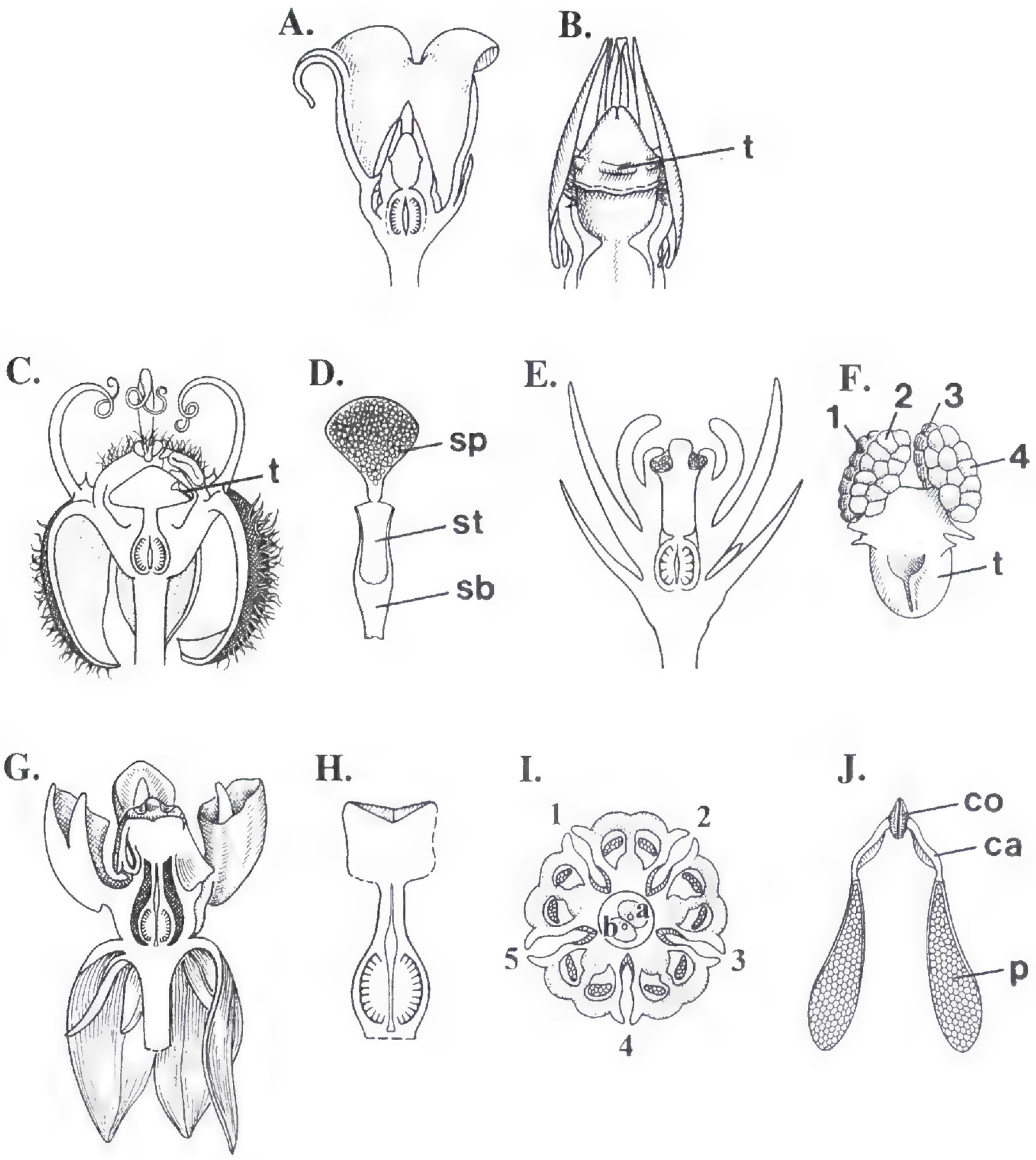

Figure 1. Flower morphology of plants selected to represent some major morphological transformations in the evolutionary history of the Apocynaceae s.l. A–B. *Apocynum* (Apocynoideae). —A. Longitudinal section, showing the two carpels that are free in the ovule-bearing region but fused above to form a style-head with a compitum. —B. Surface view of a flower from which two anthers have been removed to reveal the band-like translators (t) on the style-head. C–D. *Periploca* (Periplocoideae). —C. Longitudinal section, showing the two carpels that are free in the ovule-bearing region but fused above to form a style-head with a compitum. The translator (t) rests on the surface of the style-head. —D. A translator consisting of a stalk (st) with a sticky base (sb) and a spoon (sp) filled with pollen tetrads. E–F. *Secamone* (Secamonoideae). —E. Longitudinal section, showing the two carpels that are free in the ovule-bearing region but fused above to form a style-head with a compitum. —F. A pollinium consisting of four sacs of pollen (1–4; note that the two shaded sacs lie in a plane below the two others) and a translator (t). G–J. *Asclepias* (Asclepiadoideae). —G. Longitudinal section, showing the location of the style-head relative to the style and ovary. —H. Longitudinal section through the two carpels, which are joined only at their apices; the carpels lack a compitum. —I. Transverse section, showing the location of the stigmatic chambers relative to the styles. Chambers 1, 2, and 3 transmit pollen tubes to style (a), whereas chambers 4 and 5 transmit pollen tubes to style (b). —J. A pollinarium consisting of two sacs of pollen or pollinia (p) joined by a translator, composed of a corpusculum (co) and two caudicles (ca).

pollinia of these two subfamilies are not homologous. As in Periplocoideae, each pollinium in Secamonoideae contains four pollinia, resulting from the four pollen sacs in two thecae. There is no spoon-shaped translator; instead, a clip-like translator (often called a corpusculum) joins two pollen sacs from each of two adjacent anthers (Fig. 1E, F).

The pollinia of the Asclepiadoideae (except *Fockea* Endl.; Verhoeven et al., 2003) are structurally different from those of the Periplocoideae and Secamonoideae. Pollinia in Asclepiadoideae are typically interpreted as being composed of single grains (Verhoeven & Venter, 2001; Verhoeven et al., 2003; but see Livshultz et al., 2007) and are

surrounded by an outer wall formed of sporopollenin (Dannenbaum & Schill, 1991; Verhoeven & Venter, 2001). Furthermore, only one of the two pollen sacs per theca is fertile, so that each anther produces only two pollinia. Pairs of pollinia from two adjacent anthers are joined to a corpusculum by two flexible arms (caudicles) to form a pollinarium (Kunze, 1996; Liede, 1996; Fig. 1G–J). Pollination is accomplished when the corpusculum becomes clipped onto an insect's appendage. As the insect frees itself, it removes the entire pollinarium from the flower. Subsequently, a pollinium may be inserted into a stigmatic chamber (some Asclepiadeae) or wedged against a guide-rail (most other taxa), completing pollination. Again, each pollinium contains more than enough pollen grains to fertilize all of the ovules within an ovary (Wyatt et al., 2000), and at least for species studied thus far in *Asclepias*, a single father typically sires all of the seeds within a fruit (Broyles & Wyatt, 1990; Gold & Shore, 1995).

The presence of pollinia ensures that all of the pollen delivered to the stigmatic surface during a pollination event will be either compatible or incompatible. Based on experimental studies of *Asclepias exaltata*, incompatible pollen can usurp ovules and prevent them from developing, resulting in an aborted follicle (Broyles & Wyatt, 1993). Compatible pollinations, however, will result in follicles that contain a full complement of cross-pollinated seeds (cf. Broyles & Wyatt, 1990). When multiple pollinia were inserted to direct compatible and incompatible pollen tubes to the same ovary, fruit-set was reduced as much as 81% (Broyles & Wyatt, 1993).

Evolutionary loss of fused carpels. The extent of fusion of the carpels has traditionally been used to delimit infrafamilial groups within the Apocynaceae (Endlicher, 1841; Bentham, 1876; Schumann, 1895; Ly, 1986). Use of this character, however, has proved troublesome, as both congenital and postgenital syncarpy occur in the family (Fallen, 1985). Moreover, Sennblad and Bremer (1996) have shown that many tribes within the Rauvolfioideae defined on the basis of carpel number and fusion are paraphyletic. Phylogenetic reconstructions support the view that the most basal taxa in the Apocynaceae are apocarpous (Potgieter & Albert, 2001) and that congenital syncarpy has arisen independently several times within the Rauvolfioideae (Simões et al., 2007). Syncarpous taxa are also known from the other four subfamilies, but in all of these studied thus far, it appears that fusion is postgenital (M. E. Endress, pers. comm.).

The gynoecium of Apocynoideae typically consists of two carpels that are entirely free in the ovule-bearing region, but that undergo postgenital fusion of their upper parts to give rise to a complex new organ known as the style-head (Fig. 1A, B). This postgenital fusion of the upper part of the style produces a functional compitum (Walker, 1978; Endress, 1982; Lipow & Wyatt, 1999). Based on very limited sampling of taxa, Periplocoideae and Secamonoideae appear to retain this basic gynoecial plan (Kunze, 1991; S. Lipow, unpublished data). In these subfamilies, the transmitting tissue shares a common beginning in the style-head so that a single inserted pollinium can supply pollen tubes to both ovaries (Fig. 1C, E).

In at least some Asclepiadoideae, however, there is no functional compitum, even though postgenital fusion between the carpel apices takes place (Fig. 1G, H). This has been demonstrated in *Leptadenia* R. Br. of the Ceropegieae (Kunze, 1991) and in the following genera of the Asclepiadeae: *Asclepias* (Sparrow & Pearson, 1948; Sage et al., 1990), *Gonolobus* Michx. (Lipow & Wyatt, 1998), *Microloma* R. Br. (P. Bruyns, cited in Kunze, 1991), *Sarcostemma* R. Br. (Kunze & Liede, 1991), and *Vincetoxicum* Wolf (Kunze, 1991). In these taxa, it is also rare for both ovaries from one flower to mature (i.e., twin fruits), whereas twins are otherwise the rule in the APSA clade. In the absence of a compitum, production of twin fruits requires at least two insertions of compatible pollen into appropriate stigmatic regions, because each of the five separate stigmatic areas communicates with only one ovary (Fig. 1I). Three of the five stigmatic regions transmit pollen tubes to one ovary, and the other two stigmatic regions transmit pollen tubes to the second ovary (Sage et al., 1990). Apparently, multiple depositions of compatible pollinia on stigmatic areas leading to separate ovaries are rare, as twin fruits are uncommon in *Asclepias* (Wyatt & Broyles, 1994) and, presumably, in other taxa that lack a functional compitum.

We contend that some of the curious evolutionary trends within the Apocynaceae involving loss of carpel fusion can be explained as ways to reduce the wasteful effects of an inefficient postzygotic self-incompatibility system. Endress (1982) speculated that gynoecia with free carpels might have an advantage over syncarpous ones because ovaries in which few or no ovules have been fertilized will not consume limited resources by participating in fruit maturation. Indeed, postzygotic self-incompatibility greatly increases the likelihood of ovaries that contain only a few compatibly fertilized ovules. With syncarpy, the majority of ovaries might receive both self- and cross-pollen tubes and, in these, self-pollen will usurp some proportion of the available ovules and prevent them from developing into viable seeds. Separating the ovaries therefore provides plants with

a means to mature only those ovaries that contain a high proportion of compatibly fertilized ovules. Thus, the evolution of postzygotic self-incompatibility may have created an unusual circumstance in which apocarpy had a clear selective advantage. Noting that "syncarpy has evolved repeatedly in the angiosperms and must be highly adaptive," Armbruster et al. (2002: 657) concluded that the paucity of reversals from syncarpy to apocarpy suggests that conditions favoring apocarpy over syncarpy are indeed "uncommon," as they found only two cases other than the partial reversal in the Apocynaceae.

An even stronger case can be made that loss of the compitum in *Asclepias* and at least a few other genera of the Asclepiadoideae represents a means of avoiding some of the most wasteful consequences of late-acting self-incompatibility. A functional compitum distributes pollen tubes to all ovaries in a flower. In *Asclepias*, however, pollen from each stigmatic chamber can reach only one ovary (Fig. 1I), which reduces the likelihood of both self- and cross-pollen being delivered to any one ovary. The loss of the compitum therefore enables one ovary to mature a fruit, even if the other is aborted due to its having received a mixed load of pollen. Such mixed pollen loads appear to be quite common, with many milkweeds, especially those that spread vegetatively, displaying high rates of self-pollination (Woodson, 1930; Morse, 1982, 1994; Pleasants, 1991; Wyatt & Broyles, 1994; Gold & Shore, 1995).

Earlier Explanations

In his monograph of the genus *Asclepias* in North America, Woodson (1954: 22) noted the difficulty of explaining the evolution of pollinia and other morphological features of the milkweed flower and concluded that "entomophily, alone, is an unsatisfying generality." Darwin too was at a loss to explain the origin of pollinia, although he continued to adhere to an adaptationist view (Burkhardt & Smith, 1990). The prevailing explanation, that pollinia confer a fitness advantage by virtue of increased efficiency of pollen delivery, was voiced earlier by one of the authors (Wyatt, 1976: 851): "This greater efficiency could represent a part of the advantage accruing to asclepiads with their pollen grains being transferred as a unit." This conclusion was reached on the basis of data for *A. tuberosa* L., which showed that overall pollination efficiency was about 1.71% (i.e., for every 100 pollen grains produced, 1.71 were actually deposited on stigmas), a figure several times higher than values reported for species with loose pollen grains (Wyatt, 1976). The likelihood of selection for pollination efficiency being so strong and consistent across so many derived taxa of the Apocynaceae, however, seems remote. This is especially true given the wide range of environments, including pollinator diversity, that various Apocynaceae experience. Moreover, it is striking that, despite having "the most elaborate, complicated flowers of all the dicots" (Endress, 1994: 302) and "extreme adaptation to insect pollination" (Safwat, 1962: 95), species of *Asclepias* have unexpectedly low fruit-set compared to most other groups of flowering plants (e.g., Wilbur, 1976; Wyatt & Broyles, 1994).

The pollination efficiency argument also fails to explain why pollinia should have evolved repeatedly as a solution to low efficiency of pollen transfer only in the derived Apocynaceae (among the dicots). Certainly, lineages in other plant families have faced this challenge, and many other solutions have been explored. In the Apocynaceae, selection for mechanisms promoting increased agglutination of pollen appears to be unusually strong, as pollinia probably evolved independently at least three times in the Periplocoideae (Ionta & Judd, 2007) and at least once in the Secamonoideae–Asclepiadoideae (Livshultz et al., 2007). Moreover, the pollination efficiency argument fails to account for the apparent association of pollen aggregation and mass transfer with late-acting self-incompatibility (although we admit that the occurrence of the latter is not well studied across the family).

Another shortcoming of the pollination efficiency argument is that it is based solely on data from *Asclepias*, a highly derived genus of the Asclepiadoideae (Rapini et al., 2003). As such, it may shed little light on the forces repeatedly driving the evolution of increased agglutination of pollen in other Apocynaceae. The pollen of *Periploca aphylla* (Periplocoideae), for example, is shed as tetrads and transported in a spoon-shaped translator (Fig. 1D). This species has a much higher pollen:ovule ratio of 128:1 than that of *Asclepias*, which is less than 2:1 for most species (Wyatt et al., 2000). In fact, the pollen:ovule ratio of *P. aphylla* approaches the range of some plants with loose pollen grains (Cruden, 1977). Thus, agglutination of pollen in *P. aphylla* does not appear to increase pollination efficiency as dramatically as expected, a finding consistent with Rosatti's (1989: 454) speculation that "the mechanism of pollen transfer in the Periplocoideae is less effective than that in the Asclepiadoideae."

We believe that the greater efficiency of pollen delivery is an epiphenomenon, a fortunate happenstance of the evolution of pollinia. The driving force behind the evolution of pollinia, however, was actually selection to prevent mixed pollen loads from reaching stigmas. This more general, genetic explanation seems

to accord well with the time of origin of the structures involved and of postzygotic self-incompatibility, although our knowledge of these phenomena is very limited at the present time. In this sense, the increased pollination efficiency in *Asclepias* is a red herring. Other consequences of the morphological changes triggered by the evolution of late-acting self-incompatibility include low multiple paternity of fruits (e.g., Broyles & Wyatt, 1990, 1995; Gold & Shore, 1995) and low variation in seed number per fruit (e.g., Wilbur, 1976; Wyatt, 1977).

Rosatti (1989: 336) summed up the confusion with respect to gynoecial evolution in the Apocynaceae by noting that "the functional significance of the peculiar structure of the gynoecium (usually free but apically united in most Apocynaceae and all Asclepiadaceae) has not been clearly established." In fact, secondary apocarpy is very difficult to explain in light of the numerous advantages of syncarpy (reviewed by Endress, 1982; but see Armbruster et al., 2002). Syncarpy uses wall tissue more efficiently to enclose ovules and makes possible a wider range of fruit types than does apocarpy (Stebbins, 1974). Moreover, by allowing differentiation of a compitum, it provides for more uniform distribution of pollen tubes to ovules and maximizes pollen competition.

The evolution of an apocarpous gynoecium without a functional compitum in some Asclepiadoideae is counter to the trend toward syncarpy found in most other angiosperm lineages. Endress (1982: 48) estimated that "only about 6% of the recent angiosperm species are apocarpous." Indeed, many basal groups of angiosperms have evolved structures that function as an "extragynoecial compitum," thereby benefiting from the advantages of syncarpy while retaining the plesiomorphic state of apocarpy (Endress et al., 1983). It is therefore remarkable that the otherwise highly derived floral morphology of Apocynaceae has retrogressed by evolving carpels lacking a compitum. Kunze (1991: 250) has attempted to explain this by arguing that, because the number of pollen grains in one pollinium is limited, "an equal distribution to both ovaries would lead to many unfertilized ovules among fertilized ones." This inefficient filling of pods should be disfavored by natural selection. This interpretation, however, is problematic. Although the pollinia of some advanced Asclepiadoideae, including some (but not all) species of *Asclepias*, do not contain enough pollen grains to fertilize all of the ovules in both ovaries (Wyatt et al., 2000), we doubt that the incredibly low pollen:ovule ratios evolved before the loss of the compitum. Instead, the numbers of pollen grains per pollinium and ovules per ovary should evolve together to ensure that each pollinium contains enough pollen grains to fertilize all of the ovules in both ovaries. Kunze's (1991) argument also fails to explain how selection could drive a plant to give up a trait that allows two fruits to mature from a single pollination.

Parallel Evolution in Other Groups of Angiosperms

Many authors have noted the remarkable parallels between the Orchidaceae and the Apocynaceae, the only families in which pollinia have evolved. We doubt, however, that our explanation for the evolution of pollinia in milkweeds has any bearing on the parallel trend in orchids. Most orchid species are self-compatible (Gill, 1989), and we are not aware of any reports of late-acting phenomena in self-incompatible taxa. Rather than incompatibility mechanisms driving speciation, van Steenis (1969) has argued that orchid species evolve through the development of pollinator specificity. It is possible that the evolution of pollinia in orchids has been driven by selection to increase pollination efficiency. Certainly, a number of other characteristic features of orchids seem to be adaptations to overcome or compensate for low rates of pollination (e.g., many seeds per fruit and extremely long-lived flowers; van der Pijl & Dodson, 1966). As Stebbins (1974: 294) has argued, "Since orchid flowers are so specialized for cross-pollination that each flower is usually visited only once by a vector, a mechanism that facilitates the transport of a large number of pollen grains as a single unit would obviously have a great selective advantage in this family."

It might appear that the evolutionary trends in the APSA clade that culminate in the elegant pollinaria and the complex style-head with five stigmatic regions communicating with two apocarpous ovaries are unique and without parallel. In fact, however, there are numerous instances of parallel trends in pollen delivery and receipt that may have evolved to minimize the likelihood of mixed pollen loads or to mitigate their negative consequences. Here we discuss some of these trends in groups for which there is at least some evidence for late-acting self-incompatibility, possibly with the same genetic basis and consequences as in *Asclepias*. This list is not exhaustive, and we expect that as more attention is paid to these phenomena, additional features will prove to be explicable on the basis of preventing mixed pollen loads. We challenge experts in each of these groups to reexamine morphological character evolution in light of our explanations of androecial and gynoecial evolution in the APSA clade.

Perhaps the most directly analogous patterns to those we have outlined occur in the legumes (Fabaceae), in which self-incompatibility is common

and widespread (Arroyo, 1981). In the two subfamilies that include more tropical and woody taxa, Mimosoideae and Caesalpinioideae, more than 60% of tested species were self-incompatible, whereas among the Faboideae the figure was less than 25%. The genetic basis of self-incompatibility in the Faboideae involves a single locus that behaves in the classical gametophytic mode (de Nettancourt, 1977). There are no definitive genetic analyses of self-incompatibility in Caesalpinioideae and Mimosoideae, but there are hints that these systems do not fit the traditional model of gametophytic self-incompatibility (Sage et al., 1994). In *Acacia retinodes* Schltdl. (Mimosoideae), Kenrick et al. (1986) showed that the site of arrest of incompatible self-pollen tubes is within the nucellus. Koptur's (1984: 1134) characterization of self-incompatibility in six species of *Inga* Mill. (Mimosoideae) revealed "that the incompatibility system is gametophytic in nature and that inhibition may occur in the ovary." Late-acting self-incompatibility is also suggested by observations of other Mimosoideae and some Caesalpinioideae in crossing studies of several species of tropical trees (Ruiz-Zapata & Arroyo, 1978; Bawa, 1979; Bernhardt et al., 1984; Gibbs et al., 1999).

In *Acacia*, pollen is regularly produced in clumps termed "polyads," in which grain numbers are typically 4, 8, 16, 32, or 64 (Kenrick & Knox, 1982; Knox & Kenrick, 1983). Kenrick and Knox (1982: 721) stated that "the possession of polyads is assumed to confer a selective advantage in reproduction, since in certain asclepiads and higher orchids the composite pollen units provide an efficient means of dispersal by insects." Again, we contend that this interpretation is probably incorrect and that, instead, polyads evolved as a means of reducing the likelihood of mixed pollen loads reaching the stigmas of plants with a wasteful late-acting self-incompatibility system. Another curious feature of *Acacia* is the modification of the stigma into a cup (Kenrick & Knox, 1981), which often is of appropriate size to receive only a single polyad. Knox and Kenrick (1983) studied pollen transfer in a natural population of *A. retinodes* and found that pollinated stigmas typically received only a single polyad. We interpret the stigma cup of *Acacia* as a mechanism, like the restricted stigmatic areas of *Asclepias*, to reduce the likelihood of mixed pollen loads. Finally, the reproductive situation in *Acacia* is similar to that of *Asclepias* in that fruit-set is remarkably low (Arroyo, 1981; Bernhardt et al., 1984). We propose that this is related to high levels of geitonogamous pollen transfer and the comparatively rare event of a solitary compatible polyad reaching a stigma.

A strikingly similar set of evolutionary trends occurs in the large, tropical genus *Inga*. Koptur (1983, 1984) has shown that these trees produce pollen in polyads of 16 to 32 grains. As in *Acacia*, there is a correspondence between grain number and ovule number, and ratios of the two fall into the range 0.95–1.74. Thus, delivery of a single polyad to a stigma provides enough pollen grains to fertilize all of the ovules within an ovary. Also, as in *Acacia*, the stigmas of *Inga* are small and, although more than one polyad will fit, the number is limited (3 to 6 according to Koptur, 1983). In all seven species studied by Koptur (1984), the majority of stigma cups (53%–73%) sampled from natural populations contained only a single polyad. It is notable too that *Inga* is characterized by very low fruit-set despite "no shortage of pollination" (Koptur, 1984: 1140). Koptur (1984) attributed this to self-polyads and incompatible cross-polyads mechanically blocking access to stigmas, rather than the more insidious effects of incompatible pollen in a late-acting system of recognition and rejection.

Other evidence for parallel evolution may be found in the Ericaceae. In several of its subfamilies (e.g., Pyroloideae, Ericoideae, Epacridoideae, Empetradoideae), individual microspores fail to separate, so that entire tetrads are transported as units by pollen vectors (Stebbins, 1974; Copenhaver, 2005). This trend reaches its apogee in the subfamily Rhododendroideae, in which "pollen tetrads often are coalesced into masses by cobwebby viscin strands; the entire anther contents may then be pulled out by the pollinator and deposited on the protruding stigma of the next flower" (Zomlefer, 1994: 81). Late-acting self-incompatibility is implicated in several species of *Rhododendron* L. (Sage et al., 1994). Williams et al. (1984) reported no difference in the growth rates of self- and cross-pollen tubes, as did Padrutt et al. (1992: 656), who further postulated "some active form of self-recognition and rejection" in *R. prinophyllum* (Small) Millais. It is therefore tempting to speculate that this wasteful, late-acting self-incompatibility has selected for increased agglutination of pollen to decrease the likelihood of mixed pollen loads.

It is also possible that late-acting self-incompatibility played a role in the evolution of secondary apocarpy in the Malvales (Sterculiaceae) and the Sapindales (Rutaceae) (Endress et al., 1983), the two families outside the Gentianales in which apocarpy occurs. Late-acting self-incompatibility has been suggested for some members of these families (Sage et al., 1994). Our lack of familiarity with these taxa makes us hesitate to suggest that secondary apocarpy has evolved in these instances under the same selection pressure as in Apocynaceae, but we hope that others will evaluate this possibility more fully.

Taken as a whole, we believe that a strong case can be made linking late-acting self-incompatibility and the

evolution of certain androecial and gynoecial characters. On the male side, these include the agglutination of pollen via retention in tetrads or polyads and via coherence due to viscin threads or exinal connections. On the female side, innovations include secondary apocarpy, stigmatic receptacles for individual pollen units, and possibly sensitive stigmas that close after a single pollination event. Plants with late-acting self-incompatibility syndromes are also characterized by low levels of fruit-set (< 10%), which appear to be at least partially a consequence of the highly deleterious effects of geitonogamy and mixed pollen loads, as even the most successful adaptations are not 100% effective. Ironically, one of the reasons that late-acting self-incompatibility has proved recalcitrant to experimental genetic analysis is that plants afflicted with it have notoriously low fruit-set. In addition, researchers have been reluctant to undertake the necessary diallel crosses using long-lived woody plants. We predict that more taxa with reproductive behavior that has heretofore been enigmatic will be discovered to possess late-acting self-incompatibility. We also expect that researchers will find further examples of the sorts of adaptations we have described, which prevent mixed pollen loads or which mitigate their negative consequences.

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RESTRICTION OF POLLINATOR ASSEMBLAGE THROUGH FLOWER LENGTH AND WIDTH IN THREE LONG-TONGUED HAWKMOTH–POLLINATED SPECIES OF *MANDEVILLA* (APOCYNACEAE, APOCYNOIDEAE)[1]

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Abstract

Apocynaceae have a precise pollination mechanism. However, broad pollinator assemblages, including several insect orders, have often been recorded. We test how the corolla tube length and operative width required for effective release of the pollination mechanism could restrict the pollinator assemblage in putatively hawkmoth-pollinated Apocynaceae that differ in flower depth. If corolla tube length were effective in filtering pollinators, a broader pollen vector assemblage would be expected in short-flowered species. For this to occur, the operative width of the pollinating apparatus would be additionally expected not to be specific to any particular pollinator. Alternatively, if the pollination mechanism were to have any effect in the exclusion of pollinators, access to short flowers would be expected to be limited by proboscis width rather than length. Pollination of three species in the Apocynaceae was studied in seven populations through direct observations and palynological analysis of hawkmoths. Proboscis widths of hawkmoths carrying and not carrying pollen were compared with operative floral width as measured by proboscis analogues of different diameters that could successfully release the pollination mechanism. Species studied were exclusively hawkmoth pollinated. Pollen was always attached to pollinator proboscides, either near the base or on the apical half. The long-tongued hawkmoth species *Manduca sexta* (L.) was the major pollinator of *Mandevilla longiflora* (Desf.) Pichon and *Mandevilla petraea* (A. St.-Hil.) Pichon. Surprisingly, another long-tongued species, *Manduca tucumana* (Rothschild & Jordan), was the main pollinator of the short-flowered *Mandevilla laxa* (Ruiz & Pav.) Woodson. Here, the operative flower width was a decisive factor restricting the pollinator spectrum to hawkmoths with proboscides narrow enough to release the pollination apparatus. Short-tongued hawkmoths, which also have wider proboscides, cannot release the pollination mechanism. In *M. petraea*, the operative length, and not the operative width, restricts the pollinator assemblage. Thus, two different plant strategies were observed to restrict the pollinator spectrum: floral tube length and the operative width of the pollination mechanism.

Key words: Apocynaceae, long-tongued pollinators, *Mandevilla*, *Manduca*, operative flower width, plant-pollinator interaction, specialization, Sphingidae.

Floral structure in the family Apocynaceae received considerable attention from early botanists due to the elaborate pollination mechanism, consisting of a fertile organ complex in which anthers and stigma are coordinated or synorganized to place pollen packages precisely onto pollinators' bodies to achieve flower fertilization (Sprengel, 1793; Delpino, 1867; Endress, 1994). Most of this knowledge is based on observations of the pollination process in a few species and on broader inferences from the study of flower structure (Schick, 1980, 1982; Fallen, 1986). Although this family comprises a great number of species (ca. 4000 species) and is distributed worldwide, actual pollinator observations, i.e., visitors with pollen attached

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onto their bodies, have been reported for only approximately 120 species of Apocynaceae, indicating the need for more direct studies of pollination (Ollerton & Liede, 1997; Darrault & Schlindwein, 2005; Wiemer & Sérsic, 2006; APOPOL and ASCLEPOL databases).

The family now includes the previously recognized Asclepiadaceae (Endress & Bruyns, 2000; Potgieter & Albert, 2001), a large group composed of the subfamilies Periplocoideae, Secamonoideae, and Asclepiadoideae that is distinguished morphologically by important aspects of the construction of the pollination apparatus, which may be considered a further elaboration from the more basal subfamilies Rauvolfioideae and Apocynoideae (the Apocynaceae s. str.). Distinctive features of Asclepiadaceae include pollen dispersed in pollinia or tetrads that are provided with a translator composed of style-head secretions that have become solidified (P. Endress, 2004). In contrast, in Apocynaceae s. str. (now considered to be paraphyletic relative to the asclepiads: Potgieter & Albert, 2001; M. Endress, 2004) pollen is almost always in single grains, and the style-head secretions usually remain a viscid adhesive by which the mealy pollen adheres to the pollinators' proboscides. In this group, construction of the pollination apparatus has been studied in a considerable number of species (Demeter, 1922; Rowley, 1980; Schick, 1982; Fallen, 1986), but detailed accounts of the mechanical and dynamic aspects of its functioning have been analyzed more rarely (Demeter, 1922; Schick, 1980, 1982; Darrault & Schlindwein, 2005). Prior to and even after these studies, the dynamic aspects of the process were either incompletely or mistakenly interpreted. Schick (1982) centered his studies on the function of the pollination mechanism in two species, *Apocynum cannabinum* L. and *Vinca minor* L., which are visited (but not always pollinated) by a diverse array of insects. In this and other studies, it is not always clear whether high mechanical specificity of the floral mechanism serves to filter the sometimes broad array of visitors and narrows them to a more restricted group of actual pollinators.

In the pollination mechanism of Apocynaceae, especially members of the more derived subfamilies in which the anthers are postgenitally united with the style-head (Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae), one would expect that a considerable mechanical match is necessary between the dimensions of the pollination apparatus and those of the pollen vector, and thus, that only certain visitors with body structures mechanically suited to match the flowers can effectively function as pollinators. Interestingly, however, previous research on some Apocynaceae s. str. does not support this view, because both specialized and generalized pollinator assemblages have been observed involving pollinator species from one (Waddington, 1976; Haber, 1984; Bhatnagar, 1986; Ollerton et al., 2003; Oliveira et al., 2004) or several insect orders (Falcão & Lleras, 1981; Herrera, 1991; Ollerton et al., 2003). In addition, in a study on Asclepiadoideae, 126 species of pollen vectors were recorded on *Asclepias verticillata* L. (Ollerton & Liede, 1997).

Floral mechanisms in the Apocynaceae can be considerably versatile, and to some extent they limit utilization of floral rewards to certain visitors with suitable body geometry or behavior. For example, there are extensive records of flower visitors whose appendages become irreversibly lodged in the floral mechanism and are thus unable to pollinate (Romeo, 1933; and other references therein). This happens because of a mismatch between the width of the proboscis (or other appendage) and the width of the slit between the guide-rails (the lignified lateral sides of adjacent anthers). For such plants, the "operative width" of a proboscis can be thought of as a mechanism or strategy by which to narrow the pollinator spectrum.

In addition to operative width, a more common strategy used by Apocynaceae and many other flowering plants to narrow the pollinator spectrum has been the evolution of narrow, tubular corollas that limit flower visitors to those with mouthparts long enough to reach the nectar deep inside (Proctor et al., 1996). The most impressive of such examples are represented by plant species pollinated by long-tongued nemestrinid flies and extremely long-tongued hawkmoths (Darwin, 1862; Nilsson, 1988; Johnson & Steiner, 1997). This strategy has led to a close matching between the operative length of the flower and pollinator structures for imbibing nectar.

Within *Mandevilla* Lindl., the largest and most diverse genus of the tribe Mesechiteae, species with the sphingophilous syndrome (e.g., night-flowering, white corollas with slender lower tubes more than 10 cm long, and a strong, sweet scent) have evolved in parallel in the Northern Hemisphere (taxa previously included in the genus *Telosiphonia* (Woodson) J. Henrickson; Henrickson, 1996) and the Southern Hemisphere (taxa previously included in the genus *Macrosiphonia* Müll. Arg.; Ezcurra, 1981a, b; Galetto, 1997) and fall within two distinct clades (Simões et al., 2006, 2007a). In Argentina, four sphingophilous species with very long corolla tubes occur: *Mandevilla longiflora* (Desf.) Pichon, *M. petraea* (A. St.-Hil.) Pichon, *M. undulata* (C. Ezcurra) A. O. Simões, Kin.-Gouv. & M. E. Endress, and *M. virescens* (A. St.-Hil.) Pichon. In addition, *M. laxa* (Ruiz & Pav.) Woodson, with much shorter corolla

tubes, also exhibits the sphingophilous syndrome (Ezcurra, 1981a, b). Pollination by hawkmoths has been recorded for *M. longiflora* (Silberbauer-Gottsberger & Gottsberger, 1975) and *M. petraea* (Galetto, 1997), but pollinator identities and details of the dynamic aspects of the pollination process have remained unknown to date. Diversity of hawkmoths is considerable in Argentina, where the family Sphingidae comprises 106 species, and the range of proboscis lengths varies from 13.3 to 246.1 mm. Each biogeographical region of Argentina generally includes assemblages of both short- and long-tongued species (Moré et al., 2005). Thus, we expected that the short flowers of *M. laxa* would be pollinated by both short- and long-tongued hawkmoth species, whereas the long flowers of *M. longiflora* and *M. petraea* would be pollinated only by long-tongued moths.

To determine pollinator specificity in *Mandevilla* and to explore the potential for the two mechanisms (i.e., operative tube length and width), we studied species with the same type of pollinating apparatus but contrasting flower lengths. Our focal species were three night-blooming, hawkmoth-pollinated species of *Mandevilla* native to Argentina: *M. longiflora* and *M. petraea*, which have very long corolla tubes (> 10 cm), and *M. laxa*, with much shorter corolla tubes (about 4 cm). If corolla length effectively filters pollinators, a broader assemblage of pollen vectors would be expected in the species with shorter flowers (Fig. 1). For this to occur, one would additionally expect that the operative width of the pollinating apparatus will not be adjusted to any particular pollinator. Alternatively, if the pollination mechanism has any effect in pollinator exclusion, one would expect that in short flowers visitors with proboscides too wide to release the mechanism would be excluded (Fig. 1). To test these predictions, we considered the following aspects: (a) pollinator species involved, (b) pattern of pollen deposition on their proboscides, (c) proboscis length and width, and (d) the efficacy by which the pollination mechanism is released by actual pollinators.

Materials and Methods

STUDY AREA

Flower measurements and pollinator observations were carried out in five populations of *Mandevilla petraea* (Cuesta Blanca, CB; El Durazno, ED; El Palmar National Park, EP; San Nicolás, SN; and Unquillo, UN), one population of *M. longiflora* (Bellavista, BV), and two populations of *M. laxa* (Santa Bárbara, SB; Cuesta Blanca, CB), all located in Northern Argentina (Table 1).

LIFE HISTORY OF THE SPECIES STUDIED

Mandevilla longiflora and *M. petraea* are rhizomatous perennial herbs that grow mainly on rocky soils in arid or semi-arid grasslands. Both species have long, salverform, vespertine flowers. The corolla comprises the lobes with strongly crinkled margins and a tube which can be divided into a narrow, cylindrical lower tube and an expanded funnel-shaped throat (Fig. 1). The length of the narrow, lower tube usually varies considerably between species (e.g., in Argentina), from 6 cm in *M. undulata* to up to 13 cm in *M. longiflora* (Ezcurra, 1981a, b). Flowers open at sunset and may last up to eight nights, as in *M. longiflora* (Silberbauer-Gottsberger & Gottsberger, 1975), remaining closed during the day. Nectar volume is up to 200 µl in *M. longiflora* (Silberbauer-Gottsberger & Gottsberger, 1975), and nectar is sucrose-rich with a mean concentration of 25% sucrose equivalents in *M. petraea* (Galetto, 1997).

Mandevilla laxa is a woody liana widely distributed in the tropical Andes from Peru to northwestern Argentina. In this species, flowers have much shorter tubes (< 4 cm long) than in *M. longiflora* and *M. petraea*. Flowers last several days, remaining open during the daytime (Ezcurra, 1981a). The nectar is sucrose-rich, and the mean concentration is 27.7% (Galetto, 1997).

Mandevilla petraea and *M. laxa* have white corollas with almost identical reflectance spectra (Moré et al., 2003). Both species also have similar chemical compositions of their flower fragrances (Sérsic et al., 2004), consistent with those of other hawkmoth-pollinated species (Knudsen & Tollsten, 1993; Raguso et al., 2003a, b).

FLOWERS

Height of the anther-cone within the corolla tube (measured from tube base to anther-cone tip) and length of the corolla tube (including both the narrow lower tube and the expanded throat) were measured to the nearest 0.01 mm using a digital caliper. Flowers of *Mandevilla laxa* were inspected for scales left by hawkmoth visitors by means of a hand lens (magnification 10×), a technique that has been shown to be effective elsewhere (Nilsson & Rabakonandrianina, 1988). Fruit set of flowers open to natural pollination was recorded in one population of *M. petraea* (CB) in two different years (2000, 2002).

POLLINATORS

Hawkmoth assemblages present in the studied populations (Table 1) were determined by sampling the insects using two vertical sheet light traps (Fry &

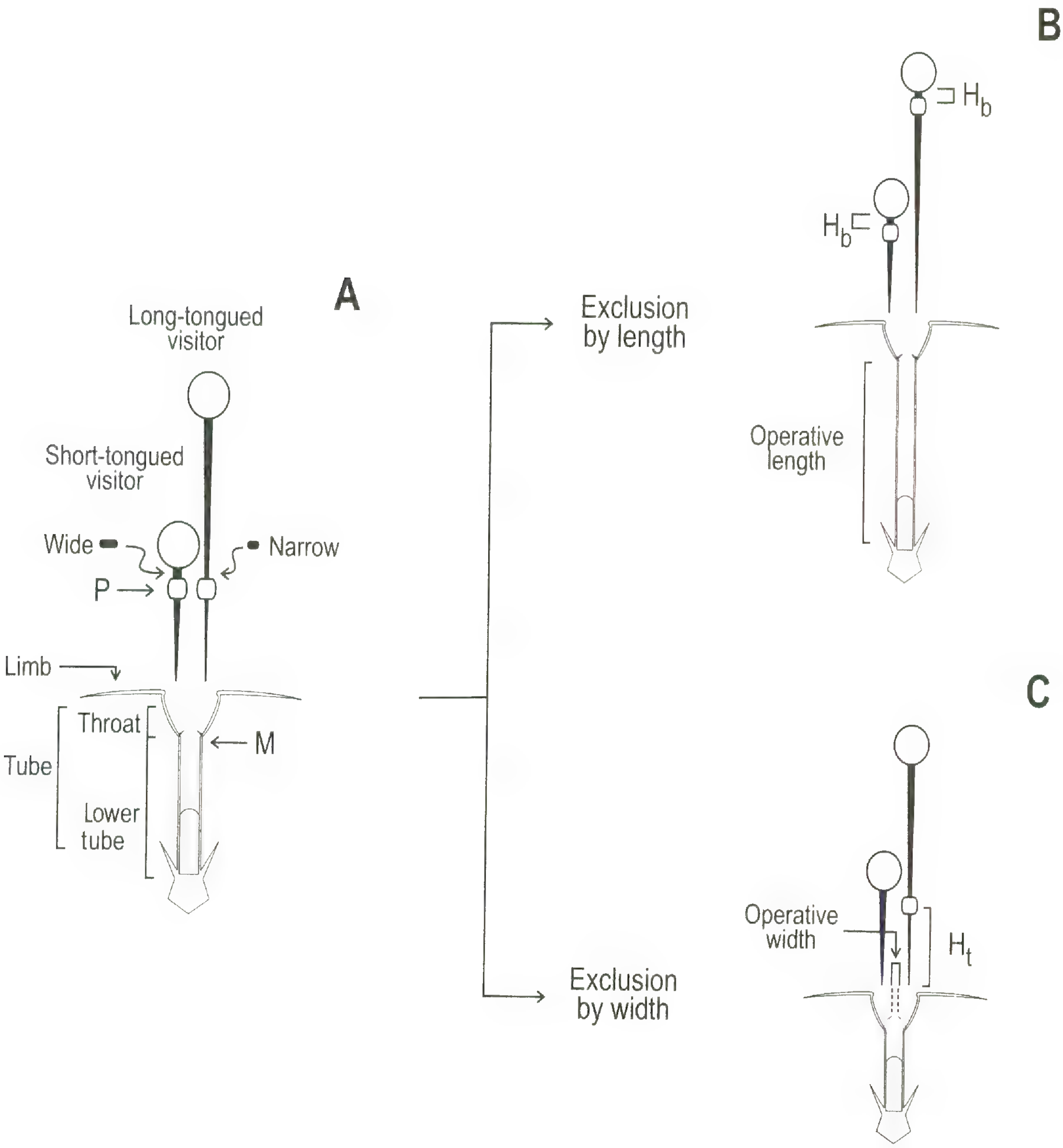

Figure 1. Hypothetical strategies for visitor exclusion in flowers of *Mandevilla*. —A. Ancestral flower type with a relatively short floral tube and a pollination mechanism (M) admitting both wide and narrow proboscides, from which short- as well as long-tongued visitors may take nectar and pollinate by extracting and depositing the pollen clumps (P), which become attached to their proboscides at a specific distance from the proboscis base (indicated as Hb in part B). —B. Hypothetical pathway leading to exclusion of visitors through increase of floral length. —C. Hypothetical pathway leading to restriction of pollinators based on the width of the pollination mechanism. Nectar is accessible to both short- and long-tongued visitors, but the entrance into the pollination mechanism is narrow, such that only visitors with a correspondingly thin proboscis are able to enter and function as a pollinator. Pollen clumps are deposited on the proboscis at a specific distance from the tip (Ht).

Waring, 2001) on a total of 14 nights. To determine the presence and location of *Mandevilla* pollen on the proboscides of the captured hawkmoths, specimens were inspected in the field under a Leica stereomicroscope (magnification 400×) (Leica Microsystems, Wetzlar, Germany). Pollen samples taken from clumps and from whole proboscides were mounted in glycerin jelly and identified under a light microscope using reference samples (Kislev et al., 1972). Direct observations of open flowers were carried out in the *M. petraea* populations totaling 22 nighttime and six daytime hours.

Proboscis length classes or guilds (irrespective of their taxonomic assignment) were assessed for the

Table 1. Populations of *Mandevilla* species studied in northern Argentina and details of hawkmoth samplings. Codes refer to locations in northern Argentina: BV = Bellavista; CB = Cuesta Blanca; ED = El Durazno; EP = El Palmar National Park; SB = Santa Bárbara; SN = San Nicolás; UN = Unquillo.

Populations	Code	Vouchers	Province	Latitude S	Longitude W	Altitude (m)	Date of hawkmoth samplings	No. of captured hawkmoths	No. of hawkmoths carrying pollen of	
									M. longiflora or *M. petraea*	*M. laxa*
M. longiflora										
Bellavista	BV	*Cocucci et al. 2537* (CORD)	Corrientes	28°30′04.6″	59°02′55.5″	66	19 Nov. 2003	9	1	
							22 Feb. 2003	34	2	
M. petraea										
San Nicolás	SN		Córdoba	31°26′58.6″	64°25′40.1″	694	27 Nov. 2002	35	1	
Cuesta Blanca	CB	*Cocucci 3905* (CORD)	Córdoba	31°24′21.6″	64°34′00.5″	820	3 Nov. 2000	54	1	
							8 Nov. 2001	88	4	
							24 Oct. 2002	49	1	
							7 Jan. 2005	7	0	
							8 Jan. 2005	7	2	
El Durazno	ED	*Cocucci 3907* (CORD)	Córdoba	31°22′03.9″	64°37′41.8″	1117	4 Mar. 2001	15	2	
National Park El Palmar	EP	*Cocucci et al. 2954* (CORD)	Entre Ríos	31°52′29.6″	58°12′36.6″	159	16 Nov. 2003	3	1	
Unquillo	UN	*Benitez-Vieyra 3906* (CORD)	Córdoba	31°14′35.9″	64°18′03.8″	605	12 Feb. 2004	3	1	
M. laxa										
Santa Bárbara	SB	*Cocucci et al. 1360* (CORD)	Jujuy	24°15′52.5″	64°26′25.2″	1370	27 Nov. 2000	117		2
							22 Feb. 2001	78		20
Cuesta Blanca	CB	*Cocucci 3908* (CORD)	Córdoba	31°28′56.8″	64°34′34.7″	793	7 Jan. 2005			
Total								499	16	22

hawkmoths captured in the *Mandevilla longiflora* (BV), *M. petraea* (CB, ED, EP, SN, UN), and *M. laxa* (SB) populations. These were calculated by ordering specimens by proboscis length and then subtracting on a $\log_{10}$ scale the proboscis length of each individual from that of the previous shortest length. The greatest difference was used to split the data into two classes, and a Kruskal-Wallis test was performed to determine whether there were significant differences between these classes. This procedure was then iterated with the next greatest difference until no new significantly different groups were found.

We tested whether the proboscis length of captured hawkmoths influenced the probability of carrying pollen of *Mandevilla* species in each population. We thus determined whether hawkmoth individuals carrying pollen occurred in a random sequence in a series of increasing proboscis lengths by means of runs tests (Sokal & Rohlf, 2000). These tests were carried out for all hawkmoths captured in the populations of each plant species studied and separately for each guild of hawkmoths carrying pollen.

Differences in proboscis width were calculated for all hawkmoth species captured in the *Manderilla petraea* and *M. laxa* populations. We expected that in the latter taxon (but not the former), floral mechanism rather than tube length would determine the specificity in pollen vectors. To detect differences in proboscis width, the insect guilds that contained individuals carrying *M. petraea* or *M. laxa* pollen were divided in two subgroups based on whether no individual of a species carried pollen or at least one individual of a species carried pollen. Proboscis width was measured in one to three specimens of each species of both subgroups at intervals of 5 mm distance from the proboscis base (Hb) for hawkmoths trapped in the *M. petraea* population, and from the proboscis tip (Ht) for hawkmoths trapped in the *M. laxa* population (Fig. 1). This was done because flowers are either longer (in the former) or shorter (in the latter) than the hawkmoth proboscides. Thus, the baseline from which to compare height of pollen deposition is respectively the pollinator's proboscis base or tip.

Measurements were made on digital photographs of the extended proboscides using the Image Tool program (Wilcox et al., 2002). Proboscis length guilds were tested with ANOVA for differences in proboscis width at each 5-mm interval of the $\log_{10}$ transformed width measures.

POLLINATION MECHANISM

Mechanical interactions between flowers and pollinators were studied from direct observations, photographs, videos, and the pollen deposition pattern on the proboscides of captured hawkmoths. In addition, two experiments using flowers of *Mandevilla laxa* and *M. petraea* were performed to determine: (1) the position along the model at which the pollen clump is attached, and (2) how the width of artificial proboscides might restrict the release of the mechanism.

In the first experiment, a straw (ca. 0.5 mm diam.) was inserted into the slit between the guide-rail of two adjacent anthers, down to the end of the corolla tube, in order to simulate a hawkmoth proboscis drinking nectar. The height on the straw at which pollen became attached during withdrawal was measured with a digital caliper. This experiment was performed in two populations of *Mandevilla petraea* (EP and UN, $n = 10$ and $n = 17$ trials, respectively) and on cultivated plants of *M. laxa* growing in the city of Córdoba ($n = 5$ trials).

The second experiment was performed on flowers of *Mandevilla laxa* ($n = 15$) and *M. petraea* ($n = 15$). A series of 10 fishing lines were introduced successively into the slit between the guide-rails in order of increasing diameter to test whether they successfully released the floral mechanism. A positive result was indicated by the pollination apparatus capturing the proboscis model. Fishing lines of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, and 1.5 mm diameter were introduced once in each of two to five guide-rail slits per flower.

RESULTS

FLOWERS

Measurements of corolla tube length and height of the style-head in the corolla tube for the three species studied are shown in Table 2. *Mandevilla longiflora* and *M. petraea* had the longest flowers, whereas in *M. laxa* they were much shorter. Both floral measurements were significantly different in relation to species (ANOVA analysis: $F_{\text{tube}} = 283.51$, $P < 0.0001$; $F_{\text{style-head}} = 449.58$, $P < 0.0001$).

POLLINATORS OF *MANDEVILLA PETRAEA*

The 261 hawkmoths captured on the *Mandevilla petraea* populations belonged to 10 species (Tables 1, 3). There was a wide variation in proboscis length, ranging from 13.30 mm in *Callionima grisescens* (Rothschild) to 123.31 mm in *Manduca rustica* (Fabricius). These hawkmoth individuals could be classified into four insect guilds (Fig. 2A) that differ significantly in their mean proboscis lengths ($H = 218.52$; $P < 0.0001$): I short, 15.50 ± 1.31 mm ($n = 23$); II medium, 30.20 ± 2.34 mm ($n = 74$); III long,

Table 2. Mean ± standard deviation (*s*) corolla tube length (including both lower tube and upper throat) and height of the anther-cone within the corolla tube of the studied species of *Mandevilla*. n = number of individuals examined; for population codes see information in Table 1.

Species	Population	Flower measurements	Mean ± *s* (mm)	n
M. longiflora	BV	corolla tube length	118.92 ± 4.69	6
		anther-cone height	113.8 ± 4.55	6
M. petraea	CB	corolla tube length	105.95 ± 7.94	13
		anther-cone height	97.79 ± 7.82	13
M. laxa	SB	corolla tube length	44.18 ± 3.34	9
		anther-cone height	32.06 ± 2.28	9
M. laxa	cultivated	corolla tube length	27.43 ± 0.61	5
		anther-cone height	21.50 ± 0.94	5

55.30 ± 4.19 mm (n = 132); and IV very long, 100.44 ± 13.74 mm (n = 31).

Mandevilla petraea pollen was carried on the proboscis of only 13 hawkmoths belonging to four insect species: *Manduca sexta* (L.) (n = 10), *Manduca diffissa* (Butler) (n = 1), *Manduca rustica* (n = 1), and *Sphinx maura* Burmeister (n = 1). The mean proboscis length of these specimens was 92.53 ± 19.40 mm (Fig. 2A), of which the most frequent pollen vector, *M. sexta*, showed a mean length of 99.04 ± 8.10 mm. These specimens belonged to the long (n = 2) and very long (n = 11) guilds (III and IV, respectively). Thus, only long-tongued and very-long-tongued specimens were acting as pollen vectors for this plant. In Figure 2A, an aggregated sequence of pollen-carrying hawkmoths is evident in the very-long-tongued guild. The runs tests showed that in a series of all hawkmoths sorted by proboscis length, the sequence of those carrying *M. petraea* pollen was not random (observed runs of 18, expected runs at 25.70; $P < 0.0001$). However, sequences were random when the series were constructed separately from hawkmoths of guilds III and IV (guild III: observed runs of 7, expected runs at 6.86; $P < 0.7710$; guild IV: observed runs of 10, expected runs at 14.33; $P < 0.0550$). Thus, the insect individuals carrying pollen were aggregated in the ordered series of all hawkmoths captured.

Pollen of *Mandevilla petraea* was always attached to the proboscis base (Fig. 3E, H). The mean height at which pollen was attached was 85.23 ± 21.14 mm from the tip or 7.30 ± 5.37 mm from the base when all specimens were included, and 92.78 ± 11.22 mm from the tip or 7.40 ± 5.88 mm from the base for the *Manduca sexta* specimens alone (Fig. 2B). Two individuals of the long-tongued guild (*Manduca diffissa* and *Sphinx maura*) were found to carry *M. petraea* pollen and clearly represent outliers from the main sequence of pollen-carrying hawkmoths.

The subgroups of hawkmoths that were or were not carrying *Mandevilla petraea* pollen were significantly different in proboscis width at a height of between 5 and 35 mm from the proboscis base (Fig. 4A). In all cases, proboscides were wider in the subgroup of long-tongued and very-long-tongued species that were carrying pollen (F_5 = 10.12, $P < 0.0155$; F_{10} = 5.37, $P < 0.0537$; F_{15} = 7.24, $P < 0.0311$; F_{20} = 7.09, $P < 0.0374$; F_{25} = 13.38, $P < 0.0106$; F_{30} = 11.16, $P < 0.0156$; F_{35} = 35.85, $P < 0.0039$).

The fruit set of labeled flowers in the Cuesta Blanca population that were left to open pollination on November 2, 2000 (n = 65), and December 27, 2002 (n = 11), was 40% and 18%, respectively.

On only one occasion could visits by hawkmoths to *Mandevilla petraea* flowers be observed accurately. A single *Manduca sexta* individual was observed to completely introduce its proboscis into the flower and, while flapping continuously, settled on the corolla limb forcing the flower to swing toward the ground (Fig. 3G). A second individual of the same hawkmoth species was photographed in midair with a pollen clump attached to its proboscis' base (Fig. 3H). No other flower visitors were observed in the studied populations of *M. petraea*. During the daytime, flowers became flaccid, as the petal lobes folded back and the corolla tube drooped toward the ground.

POLLINATORS OF *MANDEVILLA LONGIFLORA*

The 43 hawkmoths captured on the *Mandevilla longiflora* population belonged to 13 species (Tables 1, 3). There was also a wide variation in proboscis length, ranging from 18.72 mm in *Callionima inuus* (Rothschild & Jordan) to 115.77 mm in *Manduca sexta*. These hawkmoths could be classified into two guilds (Fig. 5A) that differed significantly in their mean proboscis length (H = 12.95; $P < 0.0003$): I medium, 35.74 ± 11.47 mm (n = 38); and II very long, 107.15 ± 7.78 mm (n = 5).

Mandevilla longiflora pollen was carried by only three long-tongued specimens of *Manduca sexta* (mean proboscis length 112.60 ± 3.01 mm). The runs tests of all hawkmoths sorted by proboscis length

Table 3. Number of individuals with mean and standard deviation (*s*) proboscis length by species of all captured hawkmoths from the studied populations and of those carrying *Mandevilla* pollen. For population codes see information in Table 1. Ian J. Kitching (Department of Entomology, The Natural History Museum, London, United Kingdom) helped us with the identification of the hawkmoth species. All insect vouchers are deposited at the Laboratorio de Biología Floral, Instituto Multidisciplinario de Biología Vegetal (CONICET-UNCba), Córdoba, Argentina. Note: we were unable to measure the proboscis length in one specimen of *Manduca diffissa* from CB population.

Populations	Hawkmoth species	*n*	Mean (*s*) proboscis length of captured hawkmoths (mm)	*n*	Mean (*s*) proboscis length of specimens carrying *Mandevilla* pollen (mm)
M. petraea					
CB	*Callionima grisescens* (Rothschild, 1894)	15	15.81 (1.47)		
	Hyles euphorbiarum (Guérin-Méneville & Percheron, 1835)	2	27.53 (1.92)		
	Hyles lineata (Fabricius, 1775)	3	29.63 (3.84)		
	Manduca diffissa (Butler, 1871)	56	57.76 (6.87)	1	51.69
	Manduca rustica (Fabricius, 1775)	3	114.47 (7.89)	1	123.31
	Manduca sexta (L., 1763)	12	91.98 (15.27)	5	94.73 (4.6)
	Sphinx maura Burmeister, 1879	49	53.25 (3.14)	1	52.64
	Xylophanes tersa (L., 1771)	65	30.35 (2.18)		
ED	*Erinnyis ello* (L., 1758)	1	30.83		
	Manduca diffissa (Butler, 1871)	3	58.94 (4.44)		
	Manduca sexta (L., 1763)	9	108.04 (11.82)	2	108.6 (12.12)
	Xylophanes tersa (L., 1771)	2	28.58 (6.15)		
EP	*Eumorpha labruscae* (L., 1758)	1	47.14		
	Manduca sexta (L., 1763)	1	92.92	1	92.92
	Xylophanes tersa (L., 1771)	1	30.2		
SN	*Callionima grisescens* (Rothschild, 1894)	8	14.9 (0.65)		
	Manduca diffissa (Butler, 1871)	15	56.24 (2.93)		
	Manduca sexta (L., 1763)	2	98.65 (9.66)	1	105.48
	Sphinx maura Burmeister, 1879	10	54.44 (2.65)		
UN	*Manduca diffissa* (Butler, 1871)	1	59.65		
	Manduca sexta (L., 1763)	2	106.06 (6.89)	1	101.19
Total		260		13	
M. longiflora					
BV	*Adhemarius gannascus* (Stoll, 1790)	1	26.66		
	Callionima inuus (Rothschild & Jordan, 1903)	1	18.72		
	Erinnyis crameri (Schaus, 1898)	3	38.34 (1.92)		
	Erinnyis ello (L., 1758)	2	34.97 (0.8)		
	Erinnyis impunctata Rothschild & Jordan, 1903	1	37.56		
	Erinnyis obscura (Fabricius, 1775)	16	27.31 (1.77)		
	Eumorpha fasciatus (Sulzer, 1776)	4	45.54 (1.74)		
	Eumorpha labruscae (L., 1758)	5	56.72 (2.47)		
	Hyles euphorbiarum (Guérin-Méneville & Percheron, 1835)	2	29.3 (0.86)		
	Manduca florestan (Stoll, 1782)	1	59.54		
	Manduca sexta (L., 1763)	5	107.15 (7.78)	3	112.6 (3.01)
	Xylophanes pluto (Fabricius, 1777)	1	36.75		
	Xylophanes tersa (L., 1771)	1	32.66		
Total		43		3	
M. laxa					
SB	*Callionima grisescens* (Rothschild, 1894)	14	16.33 (0.78)		
	Erinnyis ello (L., 1758)	2	35.15 (2.14)		
	Erinnyis lassauxii (Boisduval, 1859)	2	41.74 (2.90)		
	Erinnyis obscura (Fabricius, 1775)	3	26.44 (1.57)		
	Erinnyis oenotrus (Cramer, 1780)	4	42.49 (1.08)		
	Eumorpha analis (Rothschild & Jordan, 1903)	1	43.28		

Table 3. Continued.

Populations	Hawkmoth species	*n*	Mean (*s*) proboscis length of captured hawkmoths (mm)	*n*	Mean (*s*) proboscis length of specimens carrying *Mandevilla* pollen (mm)
	Eumorpha neuburgeri (Rothschild & Jordan, 1903)	2	49.02 (0.68)		
	Manduca armatipes (Rothschild & Jordan, 1916)	4	47.37 (2.15)		
	Manduca bergi (Rothschild & Jordan, 1903)	18	56.65 (2.83)	1	56.45
	Manduca diffissa (Butler, 1871)	36	65.26 (5.60)	2	64.05 (2.06)
	Manduca sexta (L., 1763)	6	102.02 (4.52)		
	Manduca tucumana (Rothschild & Jordan, 1903)	52	78.84 (3.42)	18	78.69 (2.96)
	Sphinx aurigutta (Rothschild & Jordan, 1903)	4	55.63 (14.38)		
	Sphinx maura Burmeister, 1879	37	57.48 (4.41)	1	57.75
	Xylophanes pluto (Fabricius, 1777)	1	38.33		
	Xylophanes schreiteri Clark, 1923	6	35.57 (1.23)		
	Xylophanes tersa (L., 1771)	3	32.39 (3.44)		
Total		195		22	

showed that the sequence of those carrying *M. longiflora* pollen was not random (observed runs of 2; expected runs at 6.58; $P < 0.0001$). In Figure 5A, an aggregated sequence of the three pollen-carrying hawkmoths is evident in the very-long-tongued guild.

Pollen of this species also was attached to the base of the proboscides. The mean height at which pollen was attached was 99.59 ± 8.33 mm from the tip or 12.30 ± 7.06 mm from the base (Fig. 5B). One specimen was carrying two pollen clumps that were located at different proboscis heights (Fig. 5A).

POLLINATORS OF *MANDEVILLA LAXA*

The 195 specimens captured in the *Mandevilla laxa* population belonged to 17 hawkmoth species (Tables 1, 3). There was also a wide variation in proboscis length, ranging from 15.04 mm in *Callionima grisescens* to 106.99 mm in *Manduca sexta*. These hawkmoths could be classified into three guilds (Fig. 6A) that differed significantly in their mean proboscis length ($H = 53.70$; $P < 0.0001$): I short, 16.30 ± 0.78 mm ($n = 14$); II medium+long, 62.29 ± 14.44 mm ($n = 175$); and III very long, 102.02 ± 4.52 mm ($n = 6$).

Mandevilla laxa pollen was carried by 22 long-tongued individuals (Fig. 6A) belonging to four species: *Manduca bergi* (Rothschild & Jordan) ($n = 1$), *Manduca diffissa* ($n = 2$), *Manduca tucumana* (Rothschild & Jordan) ($n = 18$), and *Sphinx maura* ($n = 1$). These species belonged to the medium+long guild (II). The mean proboscis length of these specimens was 75.53 ± 7.59 mm. The runs tests showed that in the series of all hawkmoths, and of the medium+long guild (II) only, the sequence of pollen-carrying specimens was not random (all hawkmoths: observed runs of 29, expected runs at 40.04; $P < 0.0001$; guild II: observed runs of 29, expected runs at 39.47; $P < 0.0001$). In Figure 6A, an aggregated sequence of pollen-carrying hawkmoths is evident in the right half of the medium+long-tongued guild.

Pollen of *Mandevilla laxa* was attached near the distal half of the proboscides. The mean height at which pollen was attached on hawkmoth proboscides was 27.86 ± 3.93 mm ($n = 15$) from the tip (Fig. 6B). This height could not be measured in seven specimens because pollen passed unnoticed until all pollen found on the proboscis had been microscopically identified.

Of 135 flowers of *Mandevilla laxa* inspected in the Santa Bárbara population, only seven (5%) had scales left by hawkmoths. Only one diurnal visitor was observed on flowers at the Cuesta Blanca population. This was a halictid bee that could not release the mechanism and had its proboscis stuck in the slit between the guide-rail of two adjacent anthers.

Within guild II (medium+long proboscides), the hawkmoth subgroup that was not carrying pollen included the somewhat shorter-tongued species *Erinnyis ello* (L.), *E. lassauxii* (Boisduval), *E. oenotrus* (Cramer), *Eumorpha analis* (Rothschild & Jordan), *E. neuburgeri* (Rothschild & Jordan), *Manduca armatipes* (Rothschild & Jordan), *Sphinx aurigutta* (Rothschild & Jordan), *Xylophanes pluto* (Fabricius), *X. schreiteri* Clark, and *X. tersa* (L.). The hawkmoth subgroup with pollen-carrying individuals contained the longer-tongued species *Manduca bergi*, *M. diffissa*, *M. tucumana*, and *Sphinx maura*. The hawkmoth subgroups were significantly different in proboscis width at a height of 5 mm and between 20 and 40 mm from the proboscis tip (Fig. 7A). In all cases, proboscides were wider in the subgroup that did not carry pollen ($F_5 = 5.53$, $P < 0.0351$; $F_{20} = 8.62$, $P < 0.0116$; $F_{25} = 10.17$, $P < 0.0071$; $F_{30} = 6.69$, $P < 0.0238$;

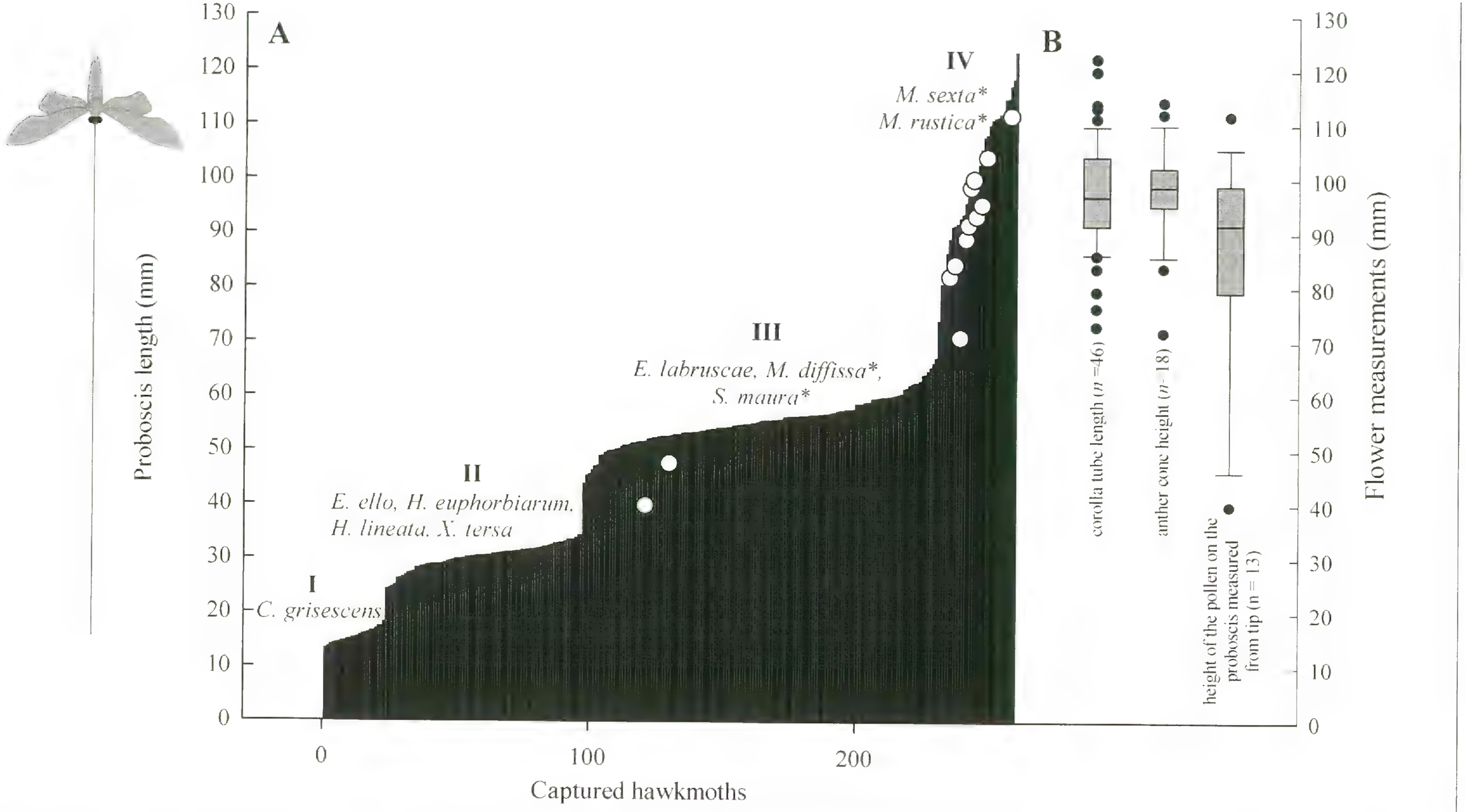

Figure 2. *Manderilla petraea*. —A. Hawkmoth guilds, guild composition, and pollen vectors in five populations (CB, ED, EP, SN, and UN). Specimens sorted by increasing proboscis length. White circles show height on the proboscis measured from the tip at which *M. petraea* pollen was attached. Asterisks represent hawkmoth species carrying *M. petraea* pollen. —B. Flower depth, as corolla tube length, and operative length, as height of the anther-cone within the corolla tube and the height of pollen clumps on the hawkmoth proboscis measured from tip. Box and whisker plots show the 10, 25, 75, and 90 percentiles for data distribution.

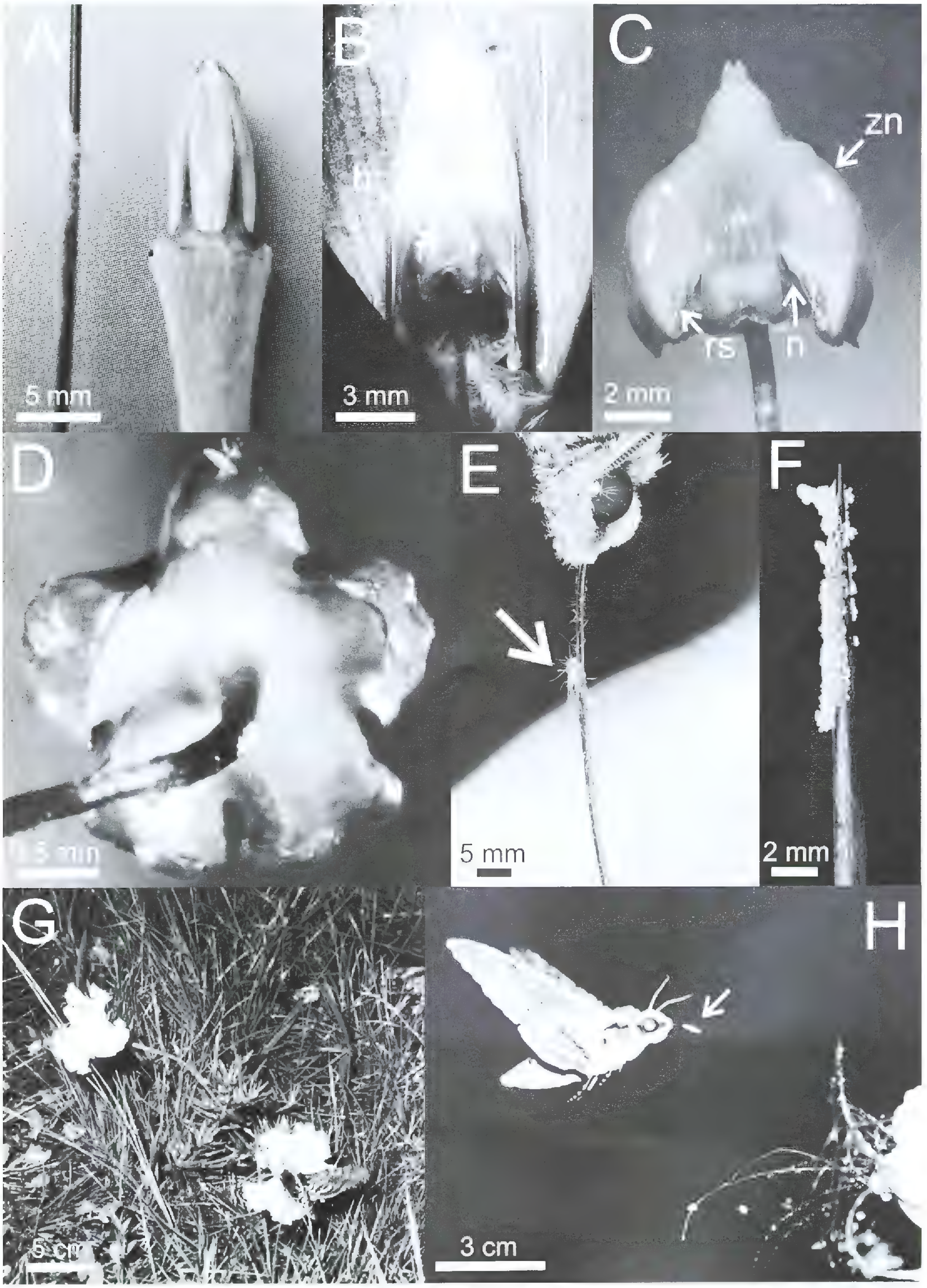

Figure 3. Floral mechanism and pollination in *Mandevilla petraea*. —A. Anther-cone situated at the top of the lower corolla tube, corolla throat removed. —B. Longitudinal dissection of the anther-cone showing the sterile (s) and fertile (f) parts of the anthers and the style-head (h). —C, D. Lateral and basal views, respectively, of the style-head showing the secretory zone (zn), notches (n), and receptive surface (rs). —E. Pollen of *M. petraea* on the proboscis base of a *Manduca sexta* male. —F. Pollen of *M. petraea* on the tip of a needle. —G. *Manduca sexta* visiting a *M. petraea* flower. —H. *Manduca sexta* visiting a *Caesalpinia gilliesii* flower (Fabaceae). Note the *M. petraea* pollen clump attached near the proboscis base (arrow).

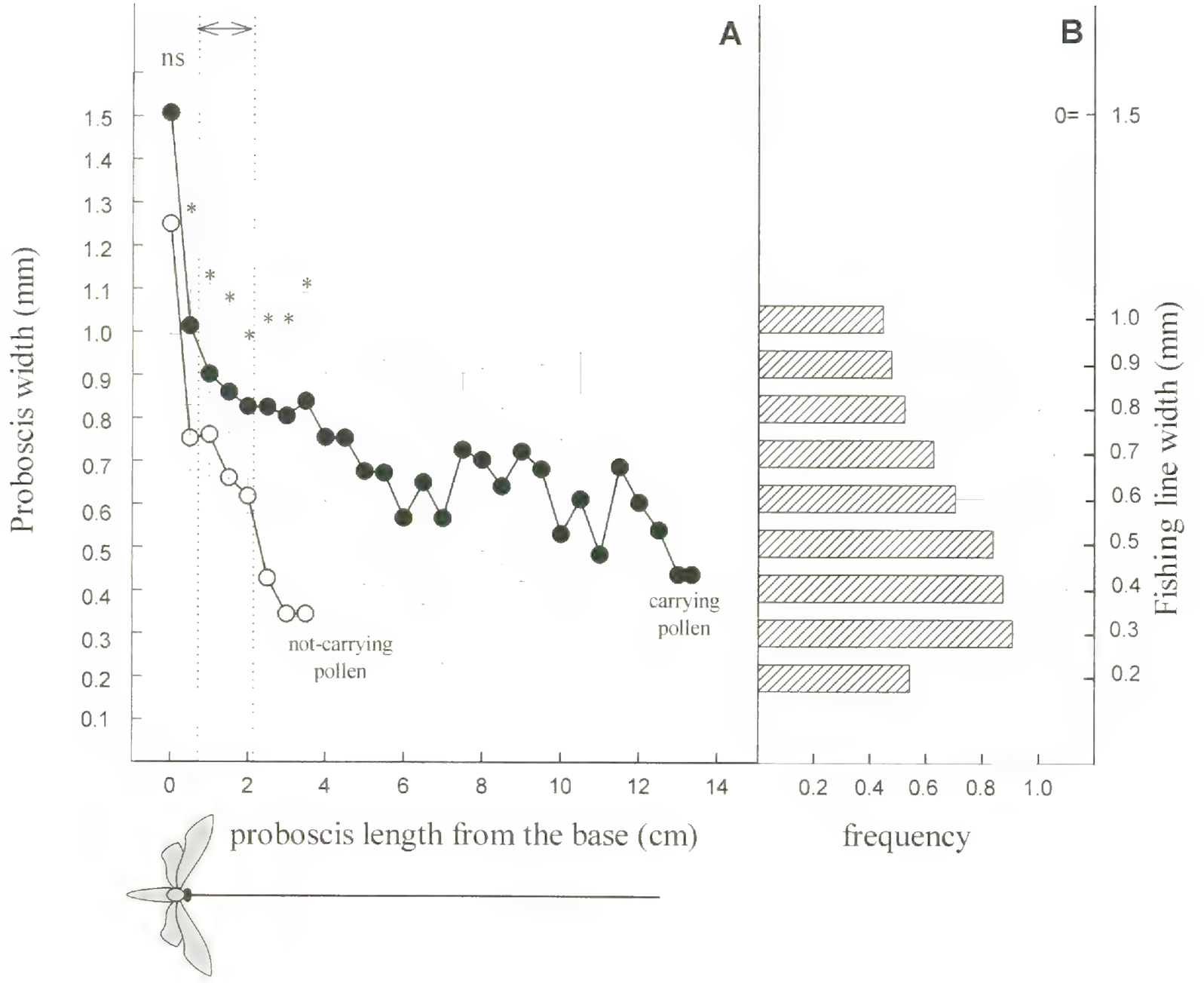

Figure 4. —A. Proboscis width measured from the proboscis base in short-tongued species that were not carrying *Mandevilla petraea* pollen (*Callionima grisescens*, *Hyles euphorbiarum*, *H. lineata*, *Erinnyis ello*, *Xylophanes tersa*, *X. schreiteri*, and *X. tersa*) and long-tongued species that were carrying *M. petraea* pollen (*M. diffissa*, *M. rustica*, *M. sexta*, and *S. maura*). The double-headed arrow at the graph top indicates the proboscis height interval in which *M. petraea* pollen was transported by long-tongued and very-long-tongued species. —B. Frequency of successful trials that released the pollination mechanism in *M. petraea* flowers. Fishing lines of 10 different diameters were used to simulate proboscides. Grey bars show mean successful trials, and error bars represent standard deviation ($n = 13$). Asterisks indicate significant differences in proboscis width between the subgroups of hawkmoths that were or were not carrying *M. petraea* pollen. See text for further details. "ns" indicates no significant differences in proboscis width between these subgroups.

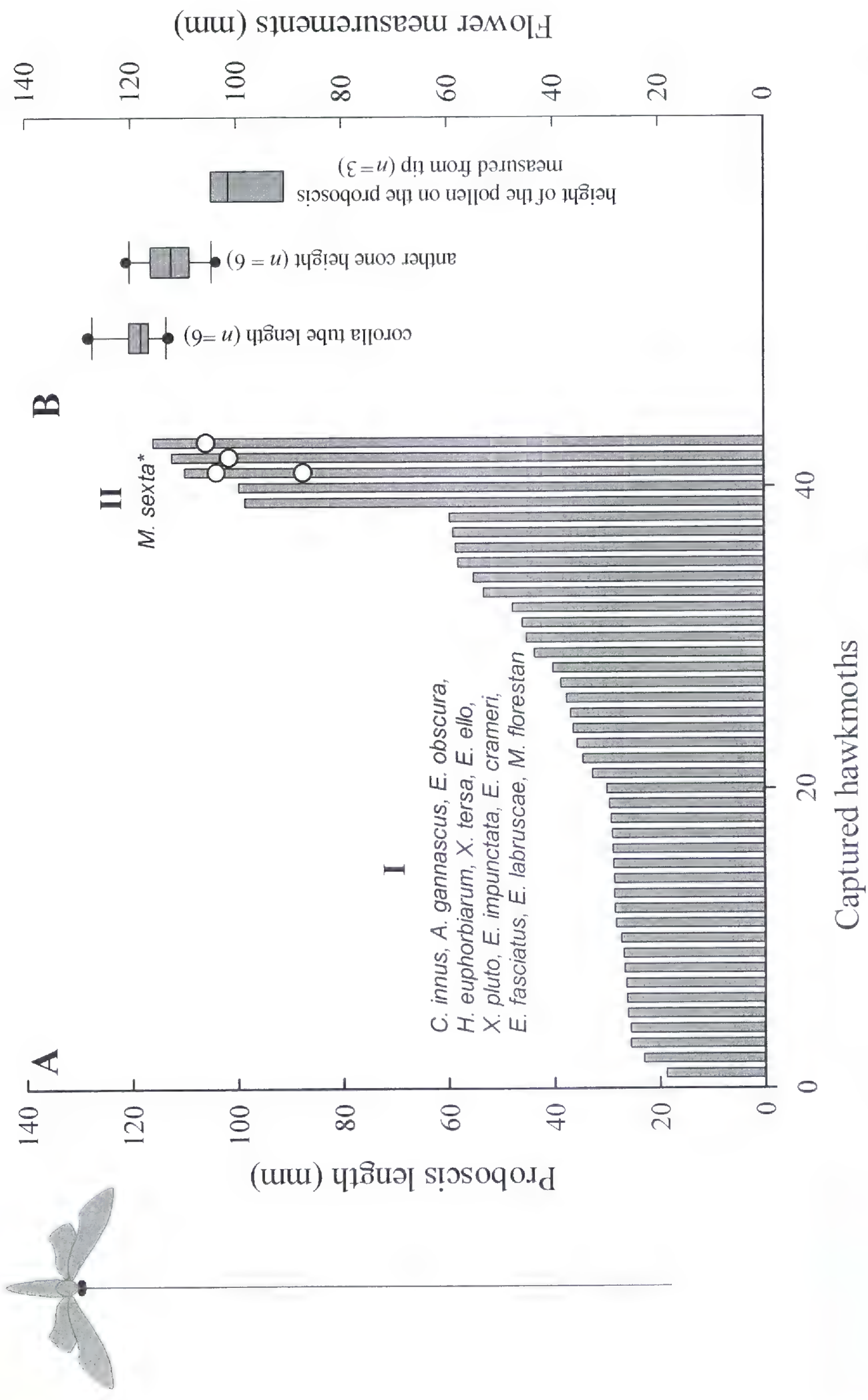

Figure 5. *Manderilla longiflora*. —A. Hawkmoth guilds, guild composition, and pollen vectors in the Bella Vista population. Specimens sorted by increasing proboscis length. White circles show height on the proboscis measured from the tip at which *M. longiflora* pollen was attached. Asterisk indicates species carrying *M. longiflora* pollen. —B. Flower depth, as corolla tube length, and operative length, as height of anther-cone within the corolla tube and height of pollen clumps on the proboscis. Box and whisker plots show the 10, 25, 75, and 90 percentiles for the data distribution.

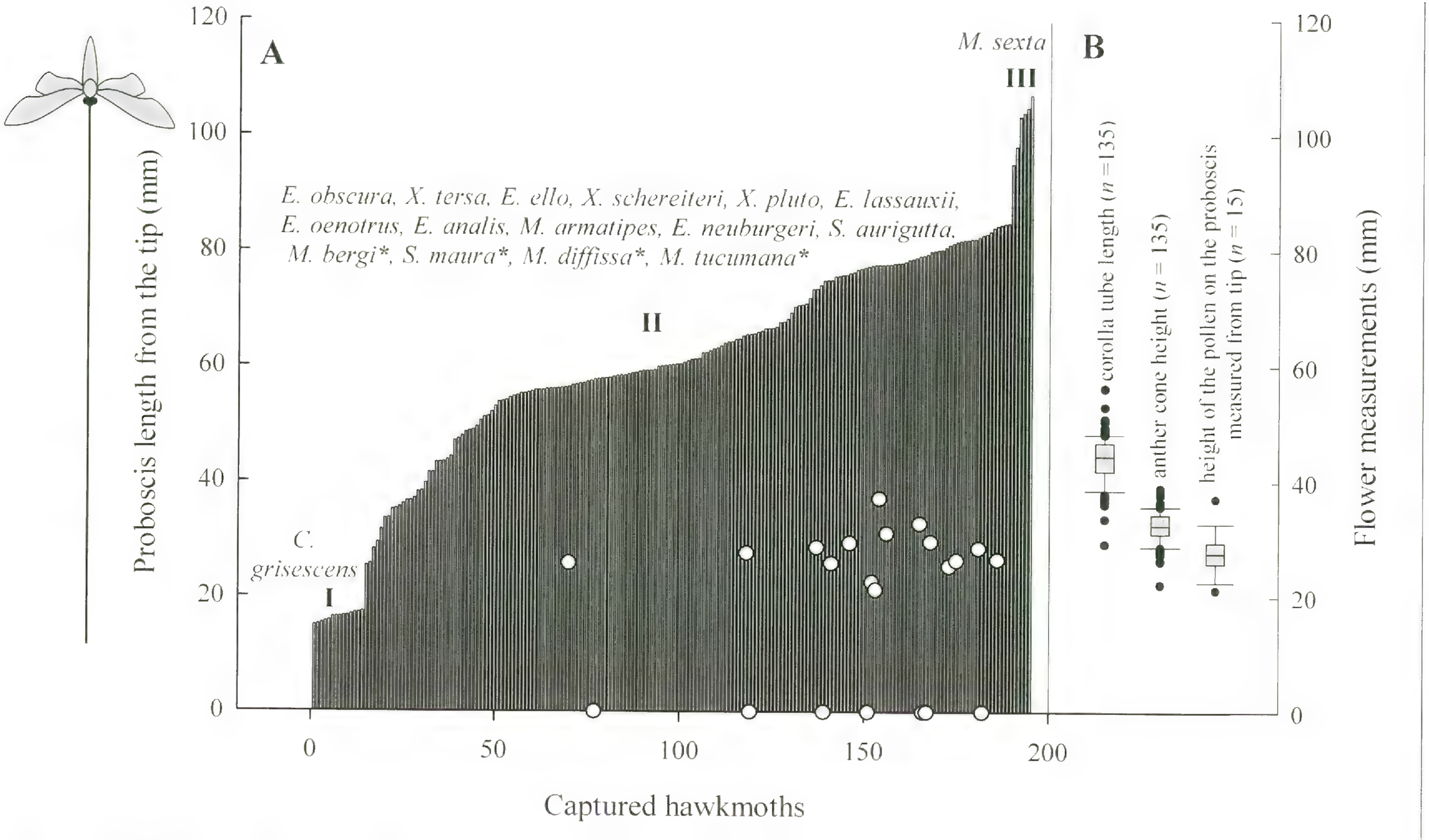

Figure 6. *Manderilla laxa*. —A. Hawkmoth guilds, guild composition, and pollen vectors in the Santa Bárbara population. Specimens sorted by increasing proboscis length. White circles show height on the proboscis measured from the tip at which *M. laxa* pollen was attached. Asterisks indicate species carrying *M. laxa* pollen. —B. Flower depth, as corolla tube length, and operative length, as height of anther-cone within the corolla tube and height of pollen clumps on the proboscis. Box and whisker plots show the 10, 25, 75, and 90 percentiles for the data distribution.

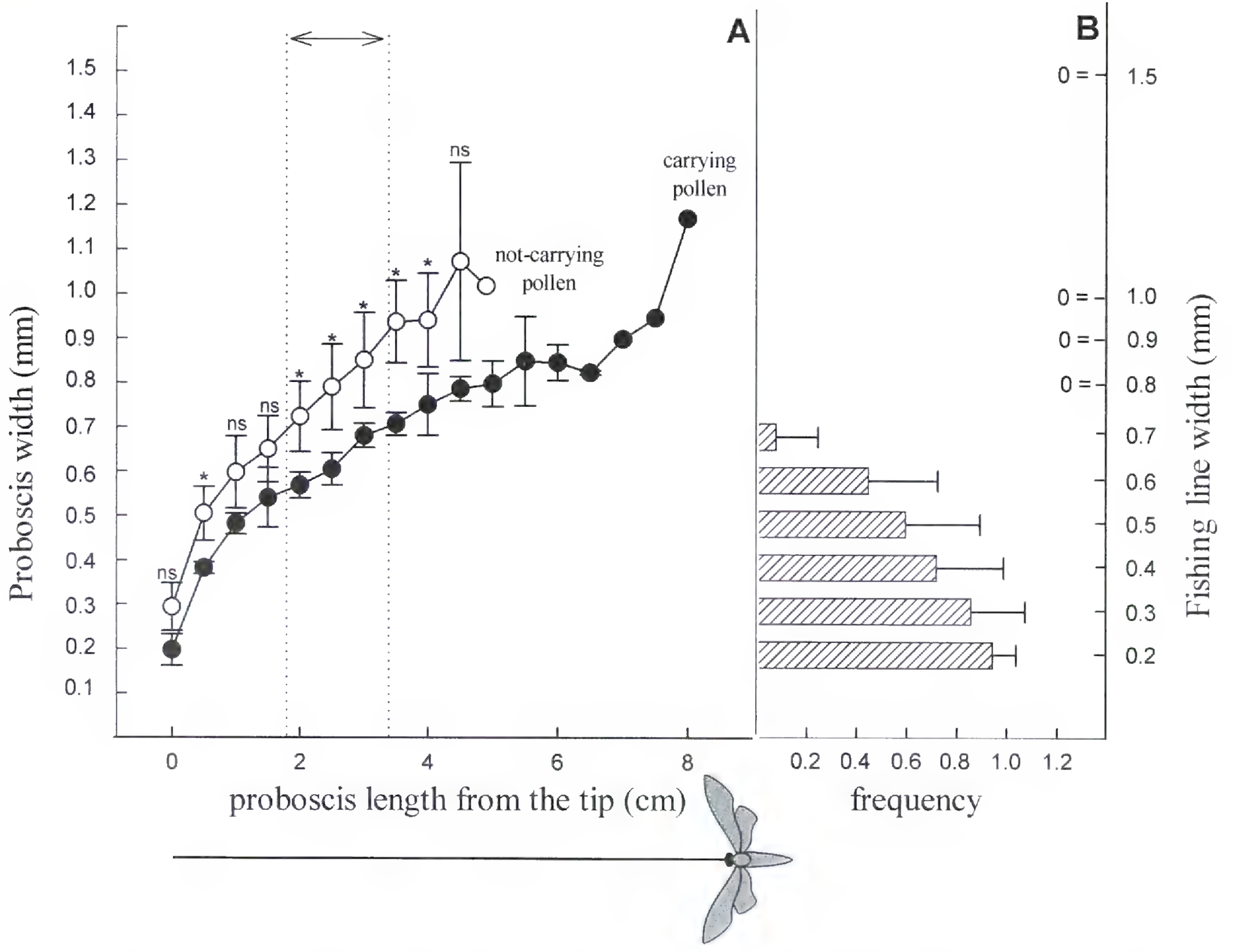

Figure 7. —A. Proboscis width measured from the tip toward the base in both short-tongued species that were not carrying *Manderilla laxa* pollen (*Erinnyis ello*, *E. lassauxii*, *E. obscura*, *E. oenotrus*, *Eumorpha analis*, *E. neuburgeri*, *Manduca armatipes*, *Sphinx aurigutta*, *Xylophanes pluto*, *X. schreiteri*, and *X. tersa*) and long-tongued species that were carrying *M. laxa* pollen (*M. bergi*, *M. diffissa*, *M. tucumana*, and *S. maura*). The double-headed arrow at graph top indicates the proboscis height interval in which *M. laxa* pollen clumps were transported by long-tongued hawkmoths. —B. Frequency of successful trials that released the pollination mechanism in *M. laxa* flowers. Fishing lines of 10 different diameters were used to simulate proboscides. Gray bars show mean successful trials, error bars represent standard deviation ($n = 14$). Asterisks indicate significant differences in proboscis width between the subgroups of hawkmoths that were or were not carrying *M. laxa* pollen. See text for further details. "ns" indicates no significant differences in proboscis width between these subgroups.

$F_{35} = 11.68$, $P < 0.0091$; $F_{40} = 7.48$, $P < 0.0291$). The largest difference between these subgroups was 0.197 mm (Fig. 7A).

POLLINATION MECHANISM

The construction and functioning of the floral pollination mechanism are essentially the same in all three species of *Mandevilla* studied. The anthers are united with the style-head, forming a complex organ, the gynostegium, located above the narrow lower corolla tube at the base of the expanded corolla throat. Anthers are lignified and connate, forming a sturdy anther-cone closely surrounding and solidly fused to the style-head. The boundaries between the guide-rails of adjacent anthers are represented by five longitudinal slits that are wider at the base and progressively narrow toward the apex, being completely closed along the apical 1/3 of the cone (Fig. 3A, B). Anther margins at the slits are sharp and curved inward. The anthers have a fertile apical part and a sterile basal part, the former corresponding to the closed region and the latter to the open region of the anther-cone slits (Fig. 3A, B). The style-head is completely enclosed within the lower part of the anther-cone and is radially differentiated, with five grooves alternating with the fused anthers, which are aligned and synorganized with the slits between the anthers, forming five longitudinal pollination chambers (Fig. 3C, D). The surface of the style-head produces an abundant slimy substance (the pollen transport adhesive; Fallen, 1986), which fills the lower region of the cone (Fig. 3B). The upper region of the cone above the style-head is radially divided into five longitudinal pollen storage chambers, each represented by the merged contents of the thecae of two adjacent anthers (Fig. 3B). Each channel opens at the tip through a soft, flexible anther acumen. The whole anther-cone is moveable by lateral tilting due to the flexibility of the short filaments subtending the anther-cone and to the thin style.

An artificial proboscis can be inserted into the corolla tube through any of the five inter-staminal spaces between the fused anthers. In so doing, the anther-cone is pushed and tilted aside. When the proboscis is withdrawn, it is caught by the base of the corresponding anther-cone slit. The inwardly curved anther slit margins act as a one-way trap, promoting upward and inward sliding along the slit through the pollination chamber. Upward traction of the proboscis opens the slits at the apical portion of the cone, which then closes back around the proboscis, sometimes with an audible click. Once the proboscis is threaded inside the pollination chamber, the anther-cone has returned to its original vertical position and the proboscis can be smoothly withdrawn. During withdrawal through the flexible anther appendages at the tip of the anther-cone, the proboscis successively slides along the concave stigmatic region beneath the expanded base of the style-head, the region filled with slimy pollen transport adhesive, and finally, the pollen chamber (Fig. 3B). Thus, pollen is attached onto the proboscis when it passes along each cone slit (Fig. 3A, F). If the proboscis was already carrying pollen, this is first deposited on the stigmatic concavity beneath the style-head.

The pollen on the hawkmoth proboscides was attached at a lower height from the tip than the height of the style-head within the corolla tube in all species. This difference, however, was significant only for *Mandevilla longiflora* and *M. laxa* (ANOVA analysis, $F_{laxa} = 34.43$, $P < 0.0001$; $F_{longiflora} = 11.83$, $P < 0.01$; $F_{petraea} = 3.45$, $P < 0.07$) (Figs. 3B, 5B, 6B). When artificial proboscides were inserted into the flowers of *M. petraea*, pollen became attached at a mean distance from the tip of 94.98 ± 6.74 mm ($n = 27$). This value did not differ significantly from that observed on the hawkmoths (ANOVA analysis, $F = 0.35$, $P < 0.71$) and was significantly lower than the height of the style-head within the corolla tube (ANOVA analysis, $F = 9.12$, $P < 0.004$). For the flowers from cultivated plants of *M. laxa*, the pollen was at a significantly lower height on the artificial proboscis 15.03 ± 0.87 mm than the style-head ($n = 5$) (ANOVA analysis, $F = 152.93$, $P < 0.0001$). This value was lower than the height of pollen on the proboscides of the hawkmoths from the Santa Bárbara population. However, we attribute this to differences between the native population and cultivated plants in height of the style-head in the corolla tube (Table 2).

In *Mandevilla petraea*, none of the trials with fishing lines 1.5 mm in diameter released the flower mechanism. Fishing lines 1 mm or thinner released the flower mechanism an average of 44%–90% of trials per flower (Fig. 4B). In *M. laxa*, none of the fishing lines thicker than 0.8 mm released the mechanism, whereas fishing lines 0.7 mm or thinner released the mechanism in 7%–94% times, and fishing lines 0.5 mm or thinner released the mechanism 60% of the time or more often (Fig. 7B).

DISCUSSION

The three *Mandevilla* species studied were pollinated exclusively by nocturnal hawkmoths. There are previous records of hawkmoth pollination for only nine species of Apocynaceae s.l., of which three belong to Asclepiadoideae (APOPOL and ASCLEPOL databases), *Cryptostegia madagascariensis* Bojer ex Decne., *Asclepias syriaca* L., and *Pergularia daemia*

(Forssk.) Chiov. Of the six species of Apocynaceae s. str., only two (*M. petraea* and *Plumeria rubra* L.) are known to be pollinated exclusively by nocturnal hawkmoths (Haber, 1984; Cocucci et al., 1992; Galetto, 1997). The four remaining species, *Carissa spinarum* L., *Hancornia speciosa* Gomes, *Nerium oleander* L., and *Vinca major* L., are pollinated by diurnal insects in addition to hawkmoths (APOPOL database: Darrault & Schlindwein, 2005). However, many putatively sphingophilous species occur within the Apocynaceae, and field observations are sorely lacking for most of them (Ollerton & Liede, 1997).

The present study demonstrates for the first time which hawkmoth species pollinate three *Mandevilla* species. Heterogeneity in flower form and color within *Mandevilla* suggests that many species do not attract hawkmoths. Indeed, bumble bees, euglossine bees, butterflies, and hummingbirds have been recorded as visitors to several species such as *M. hirsuta* (Rich.) K. Schum., *M. pentlandiana* (A. DC.) Woodson, and *M. rugellosa* (Rich.) L. Allorge (Kimsey, 1982; Torres & Galetto, 1998; Ollerton, pers. comm.). In contrast, the species that have a long corolla tube and open only at dusk, which were formerly treated as *Macrosiphonia* and *Telosiphonia*, appear to be exclusively sphingophilous (Ezcurra, 1981a, b; Henrickson, 1996), at least to the extent discernible by our examination of flower morphology, anthesis time, and pollen transfer mechanism.

Observed hawkmoth visits to the flowers of *Mandevilla petraea* were rare, even though 22 nighttime hours were dedicated to direct observation. Similarly, Silberbauer-Gottsberger and Gottsberger (1975) observed only one hawkmoth approaching the flowers of *M. longiflora* during five nights of observations. Despite this low visitation rate, the proportion of individuals setting fruit was relatively high (40% in 2000). Fruit set for *M. longiflora* also was observed to be relatively high by Silberbauer-Gottsberger and Gottsberger (1975). For *M. laxa*, visitation rate appears also to be low when estimated as the percentage of flowers with moth scales. However, this may be an underestimate of the actual visitation rate, because long-tongued hawkmoths may drink nectar without touching the corolla with their bodies.

The hawkmoths captured in populations of the plant species studied could be classified according to their proboscis length into three or four significantly different guilds. The three plant species of the present study utilized as pollen vectors only a fraction of the potential hawkmoth visitors available in the community. These hawkmoth pollinators were significantly confined in a narrow subset of all individuals sorted by proboscis length. Hawkmoths from the long-tongued and very-long-tongued guilds more often carried pollen of the short-flowered *Mandevilla laxa* and the long-flowered *M. longiflora* and *M. petraea*, respectively. The very-long-tongued *Manduca sexta* was the major pollinator (3 out of 3 in *M. longiflora* and 10 out of 13 in *M. petrea* of the pollen-carrying individuals) of both long-flowered *Mandevilla* species studied (Table 3). The long-tongued *Manduca tucumana* was the main pollinator (18 out of 22 of the pollen-carrying individuals) of *M. laxa*. The runs tests showed that within the long-tongued guild of hawkmoths visiting *M. laxa*, only the longer-tongued fraction was utilized as pollen vectors. This result was counter to our expectations and suggests that a flower mechanism other than corolla length is significant to the pollinator specificity in this species.

Apparently, through tube length alone, *Mandevilla longiflora* and *M. petraea* exclude most hawkmoths from the shorter-tongued guilds as pollinators. Pollen found on some individuals with much shorter tongues shows that they also can release the mechanism. However, their attempts to reach nectar are probably unsuccessful, which may attenuate the length of their foraging bouts or perhaps they may learn to avoid visiting these species. It was surprising to find that *M. laxa* used the long-tongued *Manduca tucumana* as its major pollinator, because its short flowers should have been accessible to the medium- and long-tongued hawkmoth guilds as well. Our data show that in this species, the operative flower width, rather than flower length per se, is the key factor in determining the type of hawkmoth pollinators that are used; thus, long-tongued pollinators are used even though flowers are relatively short. First, proboscis analogues more than 0.7 mm in diameter could not release the mechanism. Second, the mean proboscis width of short-tongued species not carrying pollen was above 0.7 mm along the segment between 20 and 40 mm from the tip and significantly wider than the long-tongued species that carried pollen. Third, pollen becomes attached to the proboscis within this range. Such a strategy, however, would not hinder some hawkmoths with short and wide proboscides to access nectar without pollinating the flowers. In conclusion, the moths with the longest tongues also had narrower tongues at the same distance from the tip than did the short-tongued species; so, those with proboscis lengths more comparable to the tube length of the flower were actually less suitable as pollinators because their tongues were too broad.

To our knowledge, only two other works have analyzed the importance of proboscis width in restricting pollen vectors. In the first, Nilsson and Rabakonandrianina (1988) hypothesized that the diameter of the spur cavity of the orchid *Aerangis*

ellisii (B. S. Williams) Schltr. would prevent some hawkmoths from fully penetrating the flower. However, they failed to demonstrate this, because even the species with the broadest proboscis fitted fully into the floral spur. In the second, Darrault and Schlindwein (2005) hypothesized that the limited fruit production in *Hancornia speciosa* Gomes, an apocynaceous species pollinated by euglossine bees, butterflies, and hawkmoths, would be due to the different number of pollen grains removed by the proboscides of its different pollinator groups. They showed proboscis width is important not in restricting pollen vectors, but in the efficiency with which it can operate the pollination mechanism. Using proboscis analogues 0.3 mm in diameter or thicker, about twice as much pollen could be extracted from flowers of this species than when analogues were 0.2 mm thick. It is important to note, however, that *Hancornia* belongs to subfamily Rauvolfioideae, in which anthers are not postgenitally united with the style-head forming a gynostegium (Simões et al., 2007b), so that the movement and diameter of the proboscis are not as restricted as in members of subfamily Apocynoideae.

Among other plant families and plant-pollinator systems, evidence exists that operative flower width may be an important adaptive character in plants with much simpler flower mechanisms. Campbell (1996) found that flower width is under selection in the hummingbird-pollinated *Ipomopsis aggregata* (Pursh) V. E. Grant, in which wider flowers show greater male fitness by exporting greater pollen quantities per visit. The mechanical explanation of this pattern is that hummingbirds can insert their bills more deeply into wide flowers and that pollen removal is greater in deep insertions. However, this mechanism does not act to narrow the pollinator spectrum, and it is not actually adjusted to the width of any body part of the pollinator. Similarly, Galen (1989) found that a greater operative flower width is favored in an almost exclusively bumblebee-pollinated *Polemonium viscosum* Nutt. population. In another mostly fly-pollinated population of *P. viscosum*, a narrower operative length is presumably associated with selection for a wider array of smaller pollinators (Galen, 1989). In contrast to these examples, the width of the slit between adjacent guide-rails in *Mandevilla laxa* acts to restrict the use of certain pollinator species. Our data partially agree with observations made in other hummingbird-pollinated plants (Temeles, 1996; Temeles et al., 2002; Castellanos et al., 2004).

The three studied species of *Mandevilla* attach pollen onto hawkmoth proboscides in the form of aggregated clumps. Pollen of *M. longiflora* and *M. petraea* was carried on the base and that of *M. laxa* on the apical half of the proboscides. The proboscis width at these positions, which was greater for *M. petraea* pollinators, agrees with the operative width of the flower mechanism as measured with proboscis analogues of different diameters. Visitors with thinner proboscides may also be captured, and if these are not strong enough to release the mechanism, they may become trapped, as did one halictid solitary bee we observed in the Cuesta Blanca population. The species studied here all have a similar pollination mechanism, in which the insect's proboscis is guided into the opening at the base of the pollen chamber by the elongate basal appendages of the anthers and becomes threaded into the slit-like one-way trap by the lignified guide-rails (Schick, 1982). The mechanism requires the proboscis to slide a short distance along the slit for it to be captured. This explains why the pollen was always deposited at a position not equal to (Silberbauer-Gottsberger & Gottsberger, 1975), but significantly lower than the height of the style-head within the corolla tube. During withdrawal from the flower, once the proboscis is captured it cannot be pulled out at the base of the anther-cone and is forced to slide its way upward through one of the five pollination chambers. Once inserted in the slit between the guide-rails, the proboscis can be then gently slid out when the anther-cone tilts back. During the process of withdrawal, stigmatic concavity, pollen adhesive, and pollen chamber are passed in that order so that pollen may be deposited onto the receptive region, then the slimy pollen transport adhesive is smeared onto the proboscis, and finally pollen is glued onto it, as hypothesized by Schick (1980, 1982) and Fallen (1986). In the three species of *Mandevilla* studied here, the floral mechanism plays an important role in determining pollinator specificity.

At the opposite extreme, the 126 visitors carrying pollen of *Asclepias verticillata* recorded by Ollerton and Liede (1997) represent an assemblage of taxonomically diverse species that may share functionally equivalent pollinating structures making them suitable for operating the floral mechanism. These guilds constitute what have recently been called "functional groups," which are more relevant to specialization than are simple species lists (Armbruster et al., 2000; Fenster et al., 2004; Wilson et al., 2004).

In our study, the nocturnal hawkmoth community varied extensively in proboscis length and width, allowing us to recognize significantly distinct functional groups. Thus, having only a selected fraction of the available hawkmoths as pollinators may have been achieved by selective mechanisms that exclude functional insect groups with proboscides either too short or too wide to release the flower mechanism. This might represent an advantage for the plant's

breeding success by reducing the chances of heterospecific pollen accumulation on the stigmas and pollen loss through incongruent deposition. Evolution of flower length has led to progressively longer operative lengths by excluding visitors with proboscides under-dimensioned for the flower length, as has already been shown in other studies (Johnson & Steiner, 1997; Nilsson, 1998).

Examples from the present study show that operative flower width excludes over-dimensioned visitors in *Mandevilla laxa*, and thus, evolution may have led to progressively thinner operative widths. Of those visitors whose proboscides are narrow enough, only those mechanically strong enough can function as pollinators, e.g., small bees were excluded as pollinators. Although we have studied only three species in the family, our study reveals novel insights toward the evolution of floral mechanisms that would be promising to address in future studies. Directional evolution of floral mechanisms in shorter-flowered *Mandevilla* and other Apocynaceae s.l. may have led the floral apparatus to adapt progressively to thinner pollinator parts, such as hairs on the insect legs or mouthparts of insects other than hawkmoths.

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POLLINATOR EFFECTIVENESS ON CO-OCCURRING MILKWEEDS (*ASCLEPIAS*; APOCYNACEAE, ASCLEPIADOIDEAE)[1]

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Abstract

Plant-pollinator interactions constitute one of the most widely recognized mutualisms, yet most investigations focus on single species or specialized pollinators. We used multiple measures to evaluate the efficiency and effectiveness of a diverse assemblage of pollinators on three co-flowering species: *Asclepias syriaca* L., *A. incarnata* L., and *A. verticillata* L. Hymenopterans exceeded other insects in their prevalence as major pollinators, but most did not vary significantly among plant species in the number of flowers visited or in visit duration per plant, except for native *Bombus griseocollis*. Significant variation in visit duration was also uncommon among insects foraging on a single taxon, except for *Apis mellifera*, which foraged longer on plants of *A. syriaca* than native hymenopterans and lepidopterans. Among the hymenoptera, bumblebees, carpenter bees, and wasps visited more plants per foraging bout, respectively, on *A. syriaca*, *A. incarnata*, and *A. verticillata*. Insect fidelity also varied seasonally and among plant species. Thus, honeybees, lepidopterans, and sphecid wasps foraged with greater relative constancy on *A. syriaca*, *A. incarnata*, and *A. verticillata*, respectively. All bees and wasps carried more pollinaria of *A. incarnata* and *A. verticillata* than of the larger-flowered *A. syriaca*, but most insects had higher inferred pollinium transfer rates on *A. syriaca* than on its congeners, especially sphecid wasps. Overall, the actions of pollinators were individualistic and indices of pollinator effectiveness based on pre-contact foraging versus vector pollinium loads were not strongly congruent. Our results highlight the need for new and creative approaches to studying the role of generalized pollination systems in the origin and maintenance of sympatrically flowering species.

Key words: *Asclepias*, common garden, foraging behavior, milkweed, pollinator effectiveness, pollinator efficiency.

Plant-pollinator interactions are widely recognized mutualisms with the potential to shape the evolution of morphological and behavioral traits. The intensity of any reciprocal adaptation between organisms will be influenced by both "quantity" and "quality" components of the interaction (Herrera, 1989); i.e., by the frequency with which a given pollinator interacts with a plant species, multiplied by its impact on fitness. To compare the relative importance of pollinators, evolutionary ecologists have sought to standardize pollinator effectiveness and efficiency measures (Thomson & Thomson, 1992; Inouye et al., 1994; Dafni & Jürgens, unpubl. data). Although researchers often estimate effectiveness of individual pollinators based on pollen transfer or fruit-set following a single visit (Inouye et al., 1994; Ivey et al., 2003; Gross, 2005), the congruence of various measures of effectiveness remains largely untested. In addition, rarely are these parameters estimated simultaneously for co-occurring plants that potentially compete for pollinator services (e.g., Dafni et al., 1997; Le Corff et al., 1998; McIntosh, 2005).

Ideally, comparative measures of pollinator effectiveness for co-occurring plant taxa would incorporate frequencies of pollinator visits, as well as data on pollen loads, patterns of pollen transfer, relative rates of pollen removal and deposition, and contributions to fruit and seed set. Pollinators that rarely visit or those with large pollen loads but little success at delivery may be less effective than pollinators with moderate visitation rates, but efficient pollen transfer (Thomson & Thomson, 1992). Behavior that leads to geitonogamy can also waste pollen resources in self-incompatible plants or reduce the effectiveness of pollination where heterozygote advantage exists (e.g., Broyles & Wyatt, 1993; Harder & Barrett, 1995). Such ineffective pollination may arise in part from inherent conflicts between behaviors that benefit the plant versus those that benefit the pollinator (Herre et al., 1999; Gregear & Laverty, 2001). Floral specialization or specialist foragers can enhance pollen transfer, but many plants are visited by generalist pollinators (Waser et al., 1996; Johnson & Steiner, 2000) or may share similar "floral syndromes" with

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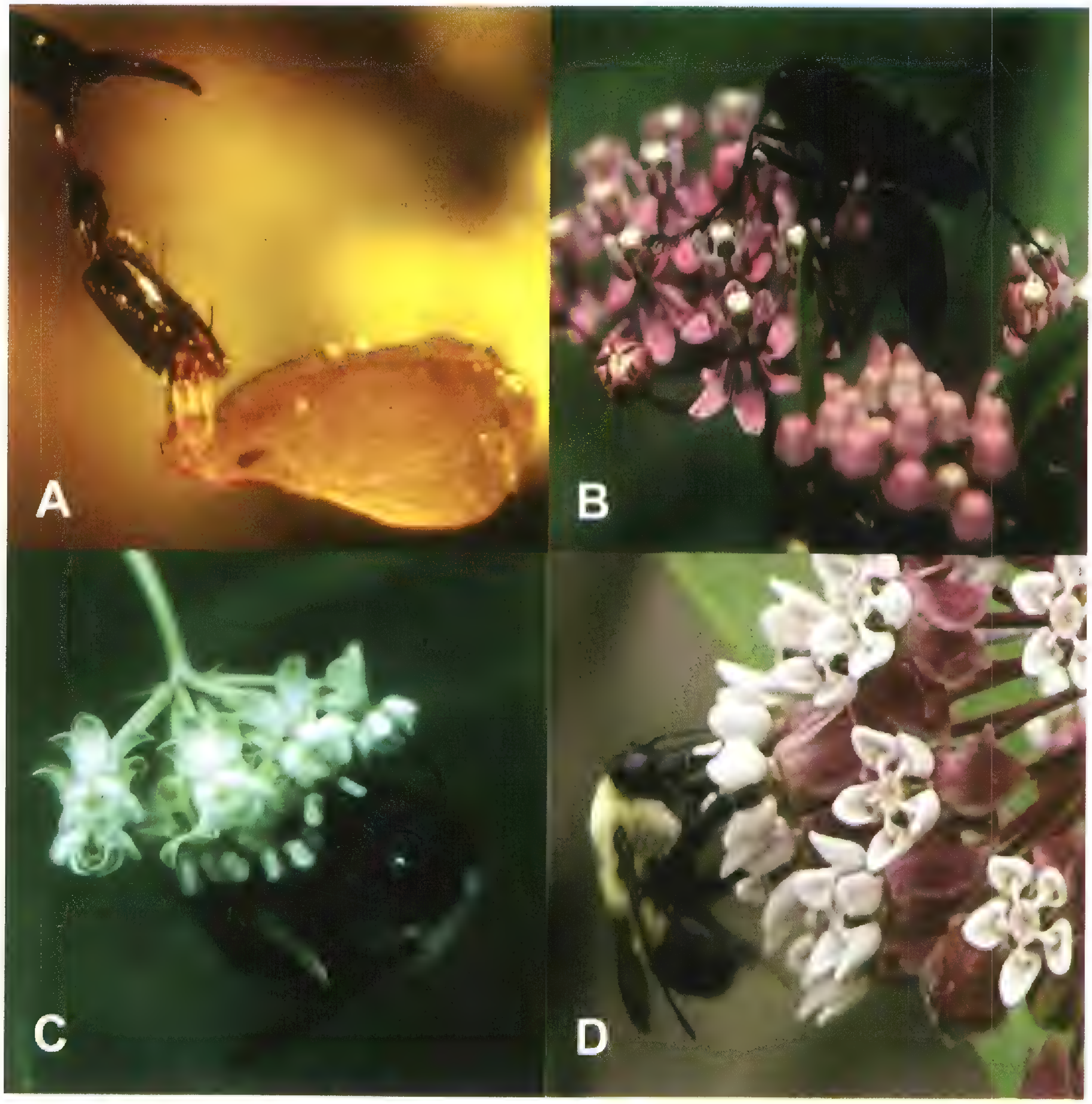

Figure 1. Pollinarium structure and major pollinators visiting three species of *Asclepias*. —A. Pollinarium of *A. syriaca* attached to corpusculum of *A. verticillata* and insect claw. -B. *Sphex pennsylvanicus* visiting *A. incarnata*. —C. *Xylocopa virginica* visiting *A. verticillata*. —D. *Bombus griseocollis* visiting *A. syriaca*.

close congeners, which might lead to partitioning of pollinator resources (Kephart, 1983).

Milkweeds (*Asclepias* L.) are particularly amenable to analyses of pollinator behavior and effectiveness in sympatric species. They are prevalent in North America, where more than 100 species with overlapping distributions are recognized (Woodson, 1954; Fishbein, 2001). The unusual flowers vary in floral architecture, yet attract diverse "generalist" visitors (Robertson, 1929; Fishbein & Venable, 1996; Ivey et al., 2003; Kephart & Theiss, 2004). The pollination system in *Asclepias* also facilitates evaluation of pollinator effectiveness. First, the pollen is packaged en masse in a pollinarium (i.e., a corpusculum joined via translator arms to two pollinia; Fig. 1). Second, insects inadvertently remove pollinaria when their appendages engage grooves in the corpuscula positioned above each of five stigmatic chambers. Moreover, corpuscula are often retained for some time on insects after delivery of the pollinia. Thus, vector pollen loads and the proportions potentially transferred to flowers can be estimated from the number of corpuscula and pollinia remaining on insect body parts (e.g., Robertson, 1929; Kephart & Theiss, 2004).

We gathered data in natural communities and a common garden to evaluate the relative effectiveness of major hymenopteran and lepidopteran polli-

nators of three co-occurring species of *Asclepias*. We define and compare multiple measures of pollinator effectiveness, including the efficiency of pollen transfer, to address the following questions. (1) *Do pollinators vary in behavior and effectiveness on diverse species of milkweeds*? For example, the relative sizes of flowers and pollinators might affect both foraging behavior and the likelihood of pollen transfer. (2) *Does pollinator effectiveness vary seasonally*? We hypothesized that foraging time on flowers, per flower visitation rates, and pollinator fidelity might vary with the extent of flowering overlap. (3) *Do different measures of pollinator effectiveness produce similar rankings of pollinators on sympatric asclepiads*? If adaptation to different pollinators exists among plant species, we predicted that multiple measures of pollinator effectiveness would produce similar outcomes.

Methods

Study Species and Populations

We used direct and indirect methods to compare the effectiveness of putative pollinators on three species of *Asclepias*: *A. syriaca* L., *A. incarnata* L., and *A. verticillata* L. These asclepiads are sympatric to parapatric in mesic prairies and disturbed sites. *Asclepias syriaca* is the most common, co-occurring near *A. incarnata* in moist areas and with *A. verticillata* in drier habitats (Woodson, 1954; Curtis, 1955). Plants flower from June to August, with variable overlap between species across sites and years, but *A. syriaca* flowers consistently ahead of its congeners (Kephart, 1987). Many of the same pollinators visit all three species (Kephart & Theiss, 2004), which differ in height, inflorescence architecture, flower size, and color (Fig. 1). The light rose to deep maroon flowers of *A. syriaca* and *A. incarnata* emit strong, sweet fragrances (e.g., linalool, *trans*-β-ocimene) that differ from the weak odor in *A. verticillata* (Dotterl et al., unpubl., Univ. Bayreuth). Plant vouchers are deposited at Chicago Botanic Garden (*A. syriaca, Paterson 1480*) and Morton Arboretum (*A. incarnata, Curtis 63402* and *A. verticillata, Swink, Betz & Lamp 7387*).

We compared pollinators on plants in diverse natural populations across the geographic ranges of the species, but predominantly in Illinois and Indiana. We also observed species in experimental arrays of equal densities ($n = 18$ same-aged plants per plot), established in a common garden (Kephart & Theiss, 2004). Although *Asclepias incarnata* and *A. verticillata* flowered in the first and subsequent summers, most *A. syriaca* plants did not flower until the second year; thus, we evaluated insect visitation to all three species in the second and third years of the study.

Measures of Pollinator Effectiveness and Efficiency

Pollinator effectiveness is used broadly to characterize any one of various measures biologists use to compare the likelihood that a given pollinator will influence plant fitness (sensu Ivey et al., 2003; Gross, 2005). Recent studies exemplify a trend to retain the useful, but problematic descriptors of pollinator "efficiency" and "effectiveness," while at the same time employing additional definitive terms (i.e., Inouye et al., 1994) for specific measurement variables (e.g., Engel & Irwin, 2003; Lau & Galloway, 2004; Gross, 2005). We computed multiple measures of pollinator effectiveness including visitation rate, time on flowers, constancy of visitation, and vector pollen load. We estimated pollen transfer efficiency based on insect capture data, as well as after single visits to virgin umbels by major pollinators, restricting the use of "efficiency" to ratios of variables that denote proportions of pollen transfer (i.e., Inouye et al., 1994). We defined potential pollinators as insects that consistently carried pollinaria and/or were observed to remove or insert pollinia.

Pollinator Behavior, Visitation, and Constancy

We differentiated three seasonal periods for which flowering sequentially emphasized the largely June flowering *Asclepias syriaca* ("early" summer), relative to *A. verticillata* and *A. incarnata*, which vary in peak flowering from "mid" to "late" summer and typically cease flowering by late August. We used two methods to record behavior, including (1) 20-minute timed observations of all visitors in experimental arrays, with movements coded as between conspecific or heterospecific plants; and (2) "sequence observations" during which we followed and timed foragers in the order encountered until they left the area or foraged > 20 minutes on a plant. Variables recorded include the duration in seconds of each visit to an umbel, the number of flowers probed and plants visited of each species, and the sequence of plants visited during each foraging bout. Observations occurred throughout the day under clear and overcast skies, as long as visitors were active. Binoculars or close inspection were occasionally necessary to confirm the identity of the insect. Unknown insects were given descriptive names, and representatives were later captured outside the plot and/or compared to existing insect collections. In data on foraging bouts, *Bombus* refers largely to *B. griseocollis*, which constituted more than 98% of individuals observed in some years. We based

rankings of visitor abundances on *Asclepias* on these foraging bouts, as well as survey walks and transects and during 10–20-minute observation periods throughout the flowering season in natural populations and experimental garden plots (Ivey et al., 2003; Kephart & Theiss, 2004).

We computed visit duration per plant based on the time an insect worked the flowers of each plant during a foraging bout, excluding grooming and other non-foraging activities. We used Kruskal-Wallis and Mann-Whitney *U* tests to evaluate the effect of insect and plant taxon (independent variables) on the number of flower visits per plant and on visit duration. We also computed the proportion of each plant species represented in bouts with visits to more than one plant taxon. Values of dependent variables were averaged for each foraging bout prior to analysis to avoid pseudoreplication.

We modeled constancy (the proportion of movements between conspecific plants) using a generalized linear model, in which visitor taxon ("insect"), plant taxon from which movement originated ("plant"), insect × plant interaction, and "season" were fixed effects. We assigned binomial errors to the models and a logit linking function (Littell et al., 1996). The response was coded as either 1 (conspecific movement) or 0 (heterospecific movement). Because of small sample sizes, all visitors in Lepidoptera were combined into a single category for these analyses ("leps"). We included data from timed observation plots and from sequence observations in this analysis of constancy. We were unable to distinguish the contributions of individual foragers to the tallied interplant movements in some cases, so each movement was treated as an independent observation. However, the vast majority of insects visited ≤ 5 plants/bout (range 2–29); only 1.4% bouts exceeded 10 plants/bout.

POLLINATOR EFFECTIVENESS AND EFFICIENCY: CAPTURE DATA

We estimated pollinator effectiveness in removing pollinaria from flowers by counting the number of attached corpuscula, and their associated pollinia (0, 1, or 2), from insects collected haphazardly from *Asclepias* flowers in common garden and natural populations. This "Vector Pollen Load" (V_E; sensu Inouye et al., 1994) estimates the total number of pollinia potentially removed and carried by major pollinators. Although V_E is affected by factors such as visitation rates, the time spent on flowers prior to capture, and grooming behavior, in some previous studies with milkweeds most hymenopterans and large lepidopteran pollinators with high pollinium loads also removed a high number of pollinaria during visits to virgin umbels (Fishbein & Venable, 1996; but see Ivey et al., 2003). We compared the pollinium load of major pollinators across plant species using a Kruskal-Wallis test.

We estimated vector pollination efficiency (Inouye et al., 1994), i.e., the efficiency of pollen transfer from an insect's body to a receptive stigma, by dividing the number of pollinia remaining on each captured insect by the total number of pollinaria on that insect. Subtracting this number from one gives an estimate of the proportion of the removed pollinia that were missing and presumably inserted by the insect. This estimate may not hold if recently attached pollinia are lost more readily or if insects carry chains of pollinaria (e.g., Morse, 1981) that may be lost en masse. However, this measure of pollination efficiency provides a point of comparison with estimates based on other methods, including those estimates derived from single visits of pollinators to virgin umbels.

All insects captured were identified to species and scored for the number and distribution of any attached pollinaria or corpuscula. We deposited insect vouchers for these specimens with Indiana University (IUIC), University of Georgia (UGCA), and with United States Department of Agriculture (USDA) laboratories in Beltsville, Maryland, in exchange for full identifications.

SINGLE VISIT POLLINATOR EFFECTIVENESS

We examined pollinium removal and insertion following single visits to virgin umbels in natural populations in Virginia and Illinois over two field seasons. We pooled data across years and across sites, under the assumption that differences among insect species in effectiveness outweighed differences between sites or between years within species. We covered individual budding umbels with bags made of organza or featherweight pellon secured with plastic-coated wire. Once flowers had opened, we allowed a single insect to forage on each umbel and recorded the duration of each visit as well as the number of flowers visited. After the visit, we collected the umbel(s) and either froze the flowers or stored them in 70% ethanol. The flowers were later dissected, examined under a microscope, and scored for the number of pollinia removed and inserted.

RESULTS

POLLINATOR BEHAVIOR, VISITATION, AND CONSTANCY

The major pollinators observed during foraging bouts on asclepiads in common garden plots included three bees (*Bombus griseocollis*, *Apis mellifera*, *Xylocopa virginica*), two sphecid wasps (*Sphex*

pennsylvanicus, *S. ichneumoneus*), and two butterflies (*Danaus plexippus*, *Speyeria cybde*). However, visitation rates varied with both insect and plant taxa. For both years, the most common visitor, *B. griseocollis*, accounted for 44%–51% and 46%–57% of the foraging bouts on *Asclepias incarnata* and *A. syriaca*, respectively, but only 7%–19% of bouts on *A. verticillata*. *Apis mellifera* visited *A. syriaca* (20%–26% of bouts) more often than other asclepiads (6%–13% of bouts), whereas carpenter bees (*X. virginica*) found on *A. verticillata* and *A. incarnata* (e.g., 13%–17%, respectively, in year 3) were rare on *A. syriaca* (0%–2% of visits). Among lepidopterans, nymphalids and papilionids occurred often on *A. incarnata* and *A. syriaca* (up to 26% of observed visits). In contrast, butterflies were infrequent on *A. verticillata* (4%–5%) and included hesperids and pierids instead.

Insects differed somewhat in their behaviors on different plant taxa. The mean number of flower visits by a particular insect taxon varied significantly with plant species only for *Bombus griseocollis* ($P = 0.01$, $df = 2$, $N = 100$ bouts, $\chi^2 = 9.3$, Kruskal-Wallis), but all major native pollinators, i.e., *B. griseocollis*, *Sphex* species, and *Xylocopa virginica*, consistently visited more flowers while foraging on *Asclepias incarnata* than on co-flowering species (Fig. 2A). In addition, *Bombus* visited more plants/bout ($\mu = 5.6 \pm 0.5$) than other major visitors to *A. syriaca* ($P < 0.001$, $df = 4$, $N = 152$ bouts, $\chi^2 = 40$). On *A. incarnata*, papilionid and nymphalid butterflies, respectively, visited the most plants/bout ($\mu = 6.7$–8.4 ± 1–3), although the mean for *X. virginica* (4.75 ± 0.8) significantly exceeded that for *A. mellifera*, *B. griseocollis*, both *Sphex* species, and *Danaus plexippus* ($P < 0.001$, $df = 7$, $N = 477$ bouts, $\chi^2 = 37$). In contrast, wasps visited more plants/bout ($\mu = 3.6$–3.9 ± 0.33–0.66) than other foragers on *A. verticillata* ($P < 0.001$, $df = 5$, $N = 126$ bouts, $\chi^2 = 19$). The number of flower visits per plant did not differ among the major insect taxa foraging on any of the plant species (Fig. 2A; $P = 0.29$–0.74, Kruskal-Wallis), but sample sizes were low for bouts with complete data for this variable ($N = 3$–58 bouts).

Visit duration in seconds varied significantly among insects foraging on *Asclepias syriaca* ($P < 0.001$, $df = 7$, $N = 208$ bouts, $\chi^2 = 26$) and *A. incarnata* ($P = 0.02$, $df = 8$, $N = 241$ bouts, $\chi^2 = 18$; Fig. 2B) but not on *A. verticillata* ($P = 0.20$, $df = 6$, $N = 105$ bouts). Non-native *Apis mellifera* exhibited the highest residence times on *A. syriaca*, whereas visits to *A. incarnata* were longest for *Bombus griseocollis*, *Xylocopa virginica*, and *Danaus plexippus* (Fig. 2B). Visit duration for a given insect varied significantly among plant species only for the high duration of *A. mellifera* on *A. syriaca* versus *A. verticillata* ($P = 0.02$, Mann-Whitney) and *B. griseocollis* on *A. incarnata* ($P = 0.03$, $df = 2$, $\chi^2 = 7.2$) relative to these same foragers on co-flowering plant congeners (Fig. 2B). For a given insect species, among-year differences also did not change the rank order among plant species; however, visits by *B. griseocollis* to *A. incarnata* and *A. syriaca* decreased in duration in the second study year (Mann-Whitney, $P = 0.001$–0.04) while visits by *Sphex ichneumoneus* to *A. incarnata* and *A. mellifera* to *A. verticillata* increased that same year ($P < 0.05$, Mann-Whitney).

The proportion of insect pollinator movements between conspecific plants in the common garden ("constancy") varied temporally ("season": $F_{2, 3919} = 78.43$, $P < 0.0001$); it was lowest early in the season (least squares mean ± standard error of the mean (SEM) = 0.66 ± 0.03), peaked mid-season (0.93 ± 0.01), and tapered somewhat toward the end of the season (0.83 ± 0.02), and all these values differed significantly from one another by Tukey-Kramer adjusted pairwise post-hoc *t*-tests ($P < 0.0001$). On average, there were no differences among plant taxa in constancy ("plant": $F_{2, 3919} = 0.49$, $P = 0.6$), suggesting that the ability of a plant to inspire fidelity was not affected by its particular floral or other traits. In contrast, insect species varied significantly in constancy ("insect": $F_{5, 3919} = 2.29$, $P = 0.04$). Specifically, *Sphex pennsylvanicus* wasps were less likely to fly between conspecifics than honeybees (*Apis mellifera*; Fig. 2C, $P < 0.05$, Tukey); other insects did not vary significantly ($P > 0.05$, Tukey). The constancy of visitors also depended on the plant taxon visited (Fig. 2C, "insect × plant": $F_{10, 3919} = 13.72$, $P < 0.0001$). These patterns suggested that visitors distinguished one plant species from the others. For example, honeybees were more likely to move conspecifically when foraging on *Asclepias syriaca*, lepidoptera had the highest constancy on *A. incarnata*, and *S. ichneumoneus* had the highest constancy on *A. verticillata* (Fig. 2C, Tukey, $P < 0.05$). In contrast, *Xylocopa virginica* were more likely to move to another taxon while foraging on *A. verticillata* (Fig. 2C, Tukey, $P < 0.05$).

POLLINATOR EFFECTIVENESS AND EFFICIENCY: CAPTURE DATA

We collected a total of 722 insects from 15 populations over three years. Insect pollinators varied significantly (Kruskal-Wallis, $P < 0.05$, $df = 19$; Fig. 3) in the number of pollinia carried (vector pollen load). *Apis mellifera* carried more pollinia of *Asclepias incarnata* and *A. verticillata* than of *A. syriaca* ($\chi^2 = 77.1$, $df = 2$, $P < 0.001$; Fig. 3), as did *Bombus* species ($\chi^2 = 81.1$, $df = 2$, $P < 0.001$), *Xylocopa virginica* ($\chi^2 = 10.1$, $df = 1$, $P < 0.001$), sphecid

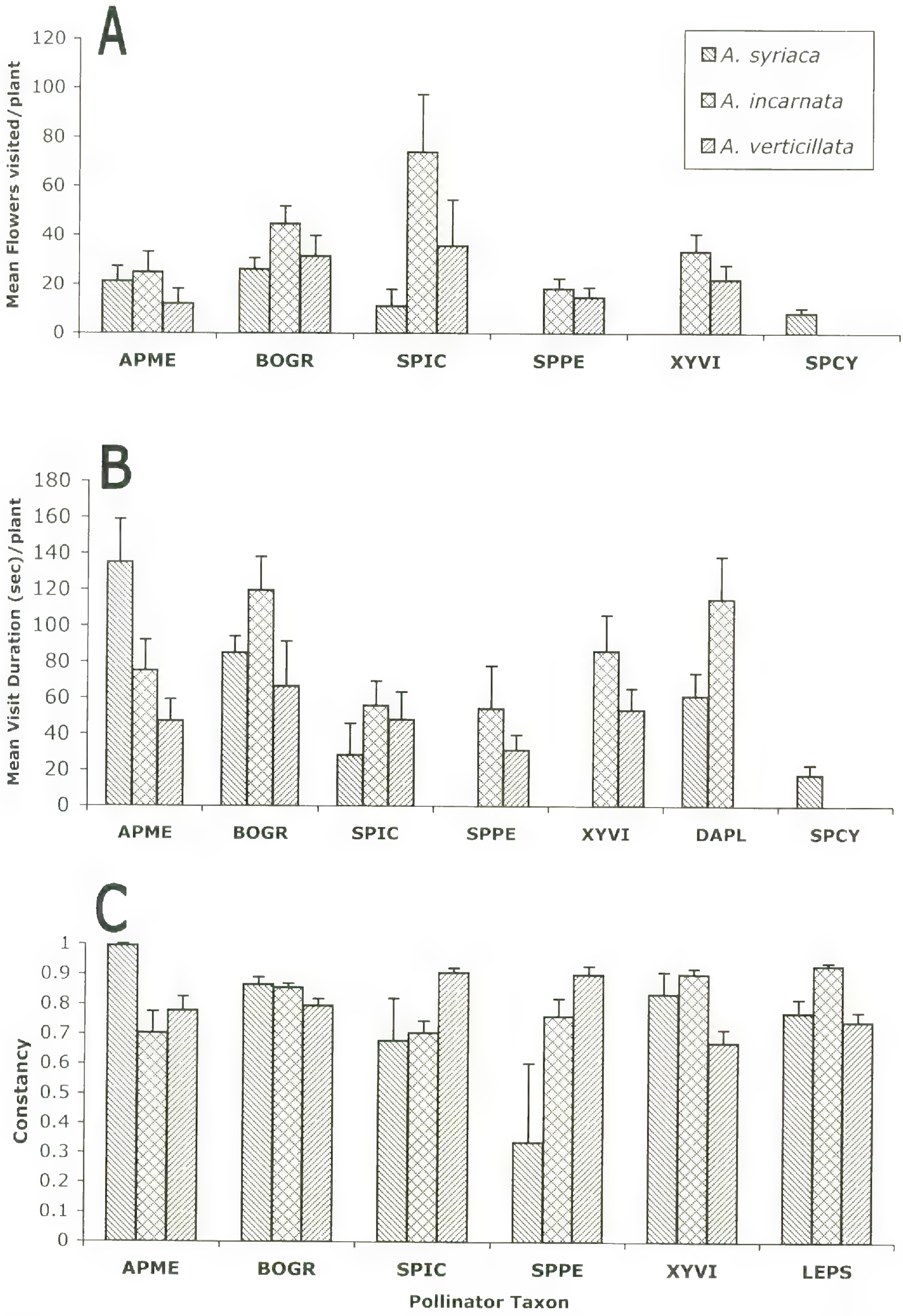

Figure 2. —A. Mean number of flowers visited per plant by the major pollinators of three *Asclepias* species growing in a common garden. —B. Mean visit duration per plant of major pollinators to three *Asclepias* species growing in a common garden. —C. Mean proportion of flights to conspecific plants (constancy) by insects foraging on three *Asclepias* species growing in a common garden. All error bars represent 1 SEM. Insect taxon codes: APME = *Apis mellifera*; BOGR = *Bombus griseocollis*; DAPL = *Danaus plexippus*; LEPS = Lepidopterans; SPCY = *Speyeria cybde*; SPIC = *Sphex ichneumoneus*; SPPE = *Sphex pennsylvanicus*; XYVI = *Xylocopa virginica*.

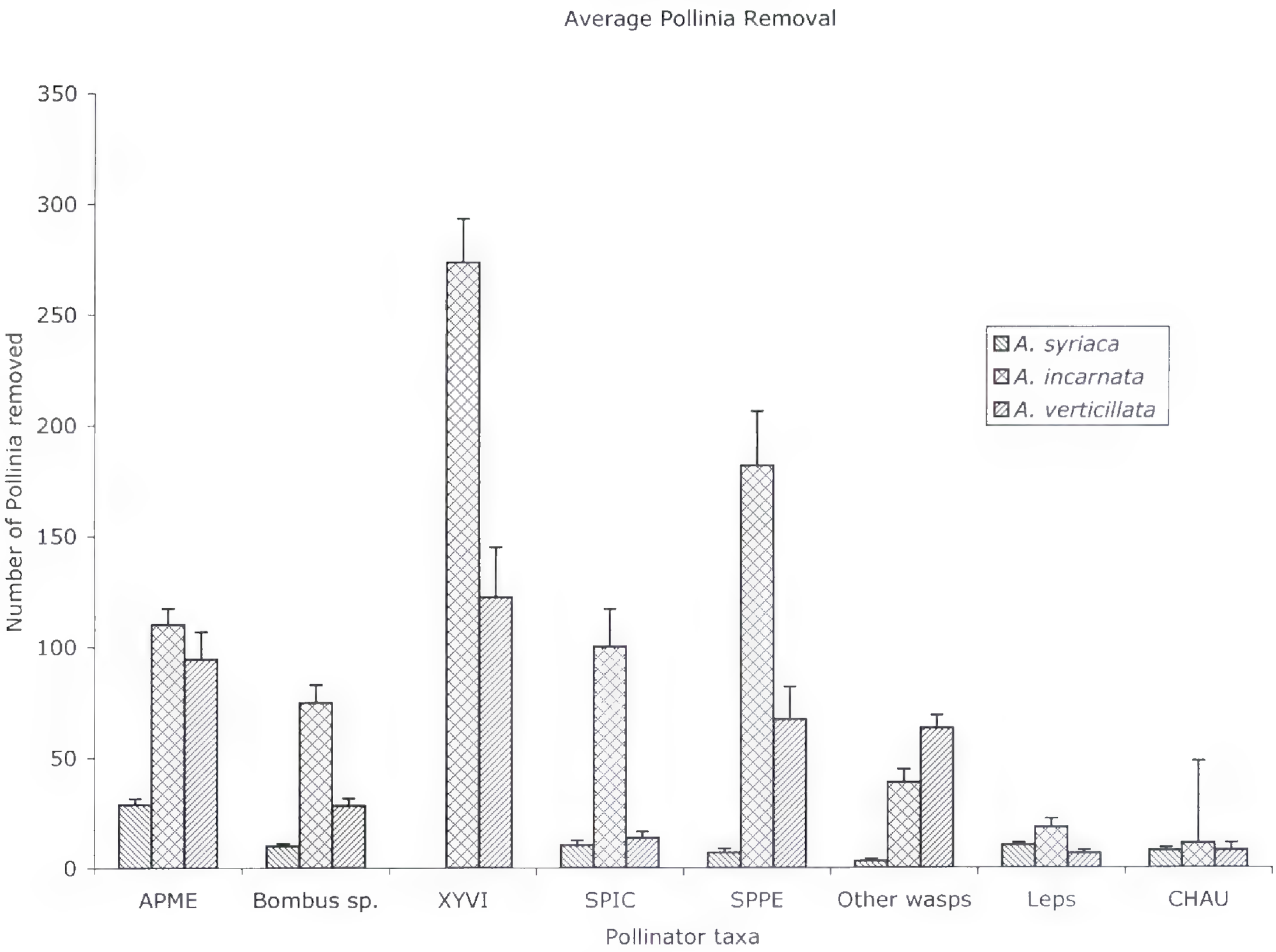

Figure 3. Estimated pollinium loads removed by insect pollinators based on the numbers of corpuscula and pollinia attached to captured visitors of three *Asclepias* species in common garden plots and natural populations. Error bars represent 1 SEM. Insect taxon codes: APME = *Apis mellifera*; CHAU = *Chaulignathus* species; Leps = Lepidopterans; SPIC = *Sphex ichneumoneus*; SPPE = *Sphex pennsylvanicus*; XYVI = *Xylocopa virginica*.

wasps (*S. ichneumoneus* and *S. pennsylvanicus*; $\chi^2 = 42.7$, $df = 2$, $P < 0.001$), and other wasps (*Myzinum* species, etc.; $\chi^2 = 16.1$, $df = 2$, $P < 0.001$). We combined all *Bombus* species to increase the total sample sizes, with *B. griseocollis* being the most common (73/97 on *A. incarnata*; 33/60 on *A. syriaca*; 39/44 on *A. verticillata*). *Xylocopa virginica* carried more pollinia of *A. incarnata* than any other taxon sampled ($P < 0.01$, $df = 6$) and more pollinia of *A. verticillata* than any insect except for *A. mellifera* ($P < 0.05$, $df = 6$). The detailed distribution of pollinia on insect bodies is provided in Kephart and Theiss (2004: table 4).

We also graphically compared estimates of the effectiveness of insects at removing pollinia (number carried corporally) with estimates of the efficiency of insect vectors in transferring pollinia to flowers (Fig. 4). The ordination of both variables in two dimensions shows several patterns evident in capture data: (1) high putative pollinium transfer efficiencies (i.e., estimated insertion effectiveness) for sphecid wasps; (2) relatively low probabilities for the potential insertion of *Asclepias verticillata* pollinia into later-visited flowers; (3) higher estimated insertion probability for diurnal lepidopterans on *A. incarnata* relative to its congeners; and (4) typically low numbers of pollinia on the bodies of insects foraging on *A. syriaca* relative to *A. incarnata* and *A. verticillata* (Fig. 4).

SINGLE VISIT EFFECTIVENESS

In both 2004 and 2005, we observed relatively few visits to *Asclepias* across all pollinator taxa (Table 1). Due to these low sample sizes and large standard errors, we did not attempt statistical analyses. *Bombus griseocollis* had the highest mean removal rate on *A. incarnata* (0.71 ± 0.35), and the day-flying hawkmoth (*Haemorrhagia diffinis*) had the lowest removal rate (*A. syriaca*, 0 ± 0, $N = 3$; *A. incarnata*, 0.03 ± 0.06; Table 1). We observed a small subset of taxa inserting pollinia (Table 1), but these events were rare.

MULTIPLE INDICES OF POLLINATOR EFFECTIVENESS

Our indices of pollinator effectiveness comprise two broad categories: (1) pre-visit measures (visitation

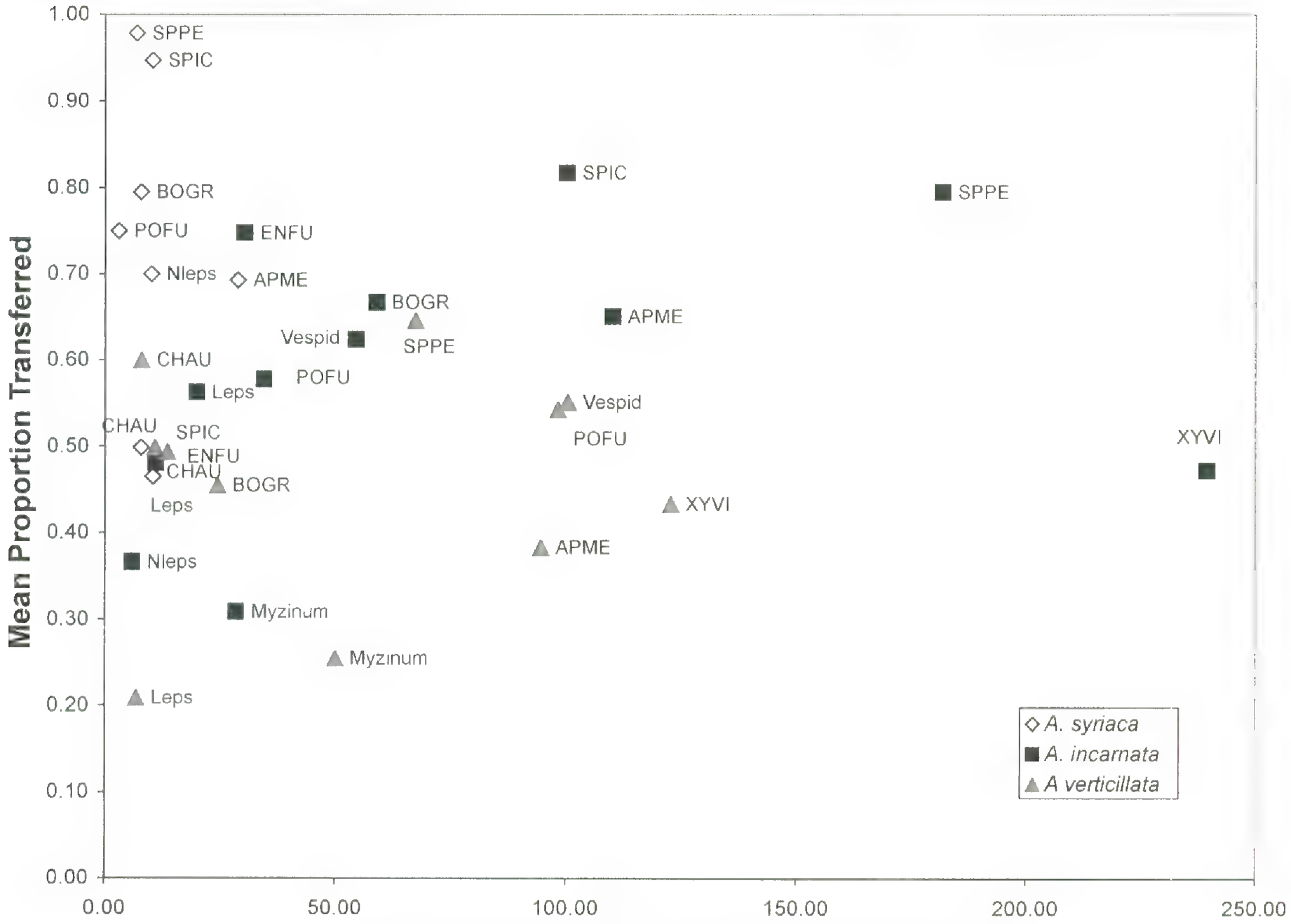

Figure 4. Ordination of mean pollinium load on captured insects relative to the estimated proportion of pollinia transferred from these same insects, presumably primarily to later-visited flowers (i.e., proportion missing from the total of all attached corpuscula × 2). Insect taxon codes: APME = *Apis mellifera*; BOGR = *Bombus griseocollis*; CHAU = *Chauliognathus* species; ENFU = *Entypus fulvicornis*; Leps = Lepidopterans; Myzinum = *Myzinum* species; Nleps = Nocturnal lepidopterans; POFU = *Polistes fuscatus*; SPIC = *Sphex ichneumoneus*; SPPE = *Sphex pennsylvanicus*; Vespid = Vespid wasps; XYVI = *Xylocopa virginica*.

Table 1. Comparisons of visitors to *Asclepias* species on experimental virgin flowers in natural populations. Numbers in parentheses following focal visitor species are the numbers of individuals observed. Pollinium removals (insertions) = mean number of pollinia removed (inserted) per flower visited (1 SEM); efficiency = mean number of insertions per removal (1 SEM).

Plant taxa	Insect taxa	Pollinium removals	Pollinium insertions	Efficiency
Asclepias syriaca	*Apis mellifera* (5)	0.253 (0.045)	0.051 (0.032)	0.200 (0.122)
	Haemorrhagia diffinis (3)	0	0	0
	Myzinum spp. (1)	0.364	0	0
A. incarnata	*Apis mellifera* (2)	0.125 (0.125)	0	0
	Bombus griseocollis (4)	0.708 (0.177)	0	0
	Haemorrhagia diffinis (3)	0.033 (0.033)	0	0
	Myzinum spp. (7)	0.465 (0.299)	0.042 (0.039)	0.108 (0.091)
	Polistes fuscatus (2)	0.395 (0.395)	0	0
	Sphex ichneumoneus (3)	0.164 (0.056)	0	0
	Sphex pennsylvanicus (12)	0.389 (0.664)	0.036 (0.019)	0.056 (0.028)
	Xylocopa virginica (3)	0.386 (0.122)	0.037 (0.037)	0.077 (0.077)
A. verticillata	*Bombus* spp. (5)	0.164 (0.065)	0.006 (0.006)	0.050 (0.050)
	Myzinum spp. (4)	0.357 (0.267)	0.119 (0.089)	0.490 (0.187)
	Sphex ichneumoneus (1)	0	0	0
	Sphex pennsylvanicus (4)	0.125 (0.066)	0	0

rate, visitation time, flowers visited, and constancy) and (2) post-visit measures (pollinium load, proportion inserted, and single visit effectiveness). Not surprisingly, varied measures yielded slightly different rank orders of pollinator taxa on each *Asclepias* species, with lepidopterans ranked as least effective overall (Table 2). Bees, including native *Bombus* species, ranked highest in pre-visit measures, especially on *A. syriaca* and *A. incarnata*, whereas wasps garnered greater importance in post-visit measures. Despite lower constancies on *A. incarnata* and *A. syriaca*, sphecid wasps exhibited high pre- and post-visit effectiveness. Non-native honeybees were most effective on *A. syriaca* and carried higher numbers of its pollinaria than some more common visitors (Fig. 3, Table 2).

Discussion

The evolution of floral divergence is often linked to specialization for particularly "effective" pollinators (sensu Stebbins, 1970), yet few investigations (e.g., McIntosh, 2005; Rymer et al., 2005) use multiple measures to compare the efficiency and effectiveness of pollinators foraging on closely related species growing in the same community. As in other studies (Willson et al., 1979; Jennersten & Morse, 1991; Ivey et al., 2003; Kephart & Theiss, 2004), a broad spectrum of hymenopteran pollinators dominated the visitor fauna of all three milkweeds, ranging in size and type from small-bodied honeybees to large native bees and sphecid or vespid wasps. Of diurnal lepidopterans, nymphalids and papilionids were more prevalent than pierids, hesperids, and hawkmoths, yet none were frequent across the entire flowering season. Except in constancy and visit duration on *Asclepias incarnata*, lepidopterans ranked low in pre- and post-visit indices for all taxa.

One important goal of this study was to identify whether the effectiveness or behavior of generalist pollinators differed on these co-occurring *Asclepias*, which vary for traits that include height, flower size, scent, and color. Pollinators differed in their approach and primary body contact on these diverse milkweeds (Macior, 1965; Kephart & Theiss, 2004), and this study extends these findings to vector pollen loads and estimated efficiencies of pollinium transfer. First, major pollinators varied markedly in the number of pollinia carried, with means ranging from 10 or fewer for hymenopterans and lepidopterans on *A. syriaca* to more than 200 for carpenter bees and nearly as many for sphecid wasps on *A. incarnata*. Vector pollinium load did not vary strictly with floral size; however, the number of corpuscula of *A. incarnata* on captured insects significantly exceeded that of both smaller-flowered *A. verticillata* and larger-flowered *A. syriaca* (Fig. 3). Despite small samples, independent estimates of pollinium removal rates from virgin flowers also were highest in *A. incarnata* after single visits by all four insect taxa for which pairwise comparisons between plant species were possible (*Apis mellifera*, *Bombus* species, *Sphex* species, and *Myzinum* species; Table 1). These results are also consistent with the high frequency of foraging bouts detected on *A. incarnata*.

Second, as evident from the ordination of estimates of pollinia removal and insertions (Fig. 4), pollinators visiting *Asclepias syriaca* overall had a higher proportion of pollinia missing from the attached corpusculum of each pollinarium. If pollinia from each species are lost at the same rate through grooming and other behaviors, these data suggest that while pollinators may remove fewer pollinia from *A. syriaca* than other sympatric asclepiads, they are more efficient at inserting them into subsequently visited flowers relative to the other two species. This potential for increased effectiveness may be associated with the position of attachment: the pollinia of *A. syriaca* attach preferentially to the aroliar pad located between the claws of all major pollinators while the tarsal hairs are the primary attachment sites for *A. incarnata* and *A. verticillata* (Morse, 1985; Kephart & Theiss, 2004; for *A. solanoana* Woodson, Lynch, 1977). The differential positioning of pollinia, combined with the apparent increased efficiency of insertion of *A. syriaca* pollinia, implies the potential for niche partitioning of pollinium attachment sites on the vector body. Studies of orchids, which also have pollinia, suggest that species sharing a common pollinator differentially place pollinia on multiple parts of the insect body, thereby maximizing pollinator effectiveness and minimizing hybridization (Nilsson et al., 1987; van der Pijl & Dodson, 1966). A broader survey of asclepiads would allow exploration of the mechanistic factors that facilitate the aroliar attachments of the larger *A. syriaca* pollinia.

We also studied behaviors that potentially influence pollinator effectiveness, including the per-flower visitation rate (i.e., related to handling time from a foraging perspective), pollinator constancy (sensu Gregear & Laverty, 2001), and two measures potentially affecting geitonogamy (the duration of visits to plants and the number of plants visited per foraging bout). The large number of *Asclepias incarnata* flowers visited/time for *Bombus* species, *Sphex* species, and *Xylocopa virginica*, relative to other flower types, is consistent with the high pollinaria loads of *A. incarnata* on insects and a higher estimated effectiveness by insects in removing its pollinia from virgin flowers. Another explanation yet

Table 2. Taxa placed in rank order for each measure of effectiveness. Relative importance = product of pollinator frequency and insect density/plot as calculated in Kephart and Theiss (2004); visitation time = mean time spent per plant (Fig. 2B); no. of flowers visited = mean number of flowers visited (Fig. 2A); constancy data based on Figure 2C; pollinium removal = estimate of pollinia removed based on counts of corpuscula on captured insects (Fig. 3); proportion inserted obtained from Figure 4; single-visit data derived from Table 1. Insect taxon codes as in Figure 4. HADI = *Haemorrhagia diffinis*.

	Pre-visit measures of pollinator effectiveness				Post-visit measures of pollinator effectiveness		
Plant taxa	Relative importance	Visitation time	No. of flowers visited	Constancy	Pollinium removal	Proportion inserted	Single visit
Asclepias syriaca	*Bombus* spp.	APME	*Bombus* spp.	APME	APME	SPPE	*Myzinum* spp.
	APME	*Bombus* spp.	APME	*Bombus* spp.	SPIC	SPIC	APME
	Leps	DAPL	SPIC	XYVI	*Bombus* spp.	*Bombus* spp.	HADI
	All wasps	SPIC	SPCY	Leps	Leps	Nocturnal Leps	
		Leps		SPIC	*Chaulignathus* spp.	APME	
				SPPE	SPPE	*Chaulignathus* spp.	
					Non-sphecid wasps	Leps	
A. incarnata	*Bombus* spp.	*Bombus* spp.	SPIC	Leps	XYVI	SPIC	*Bombus* spp.
	XYVI	DAPL	*Bombus* spp.	XYVI	SPPE	SPPE	*Myzinum* spp.
	Leps	XYVI	XYVI	*Bombus*	APME	APME	POFU
	All wasps	APME	APME	SPPE	SPIC	*Bombus* spp.	SPPE
	APME	SPIC	SPPE	APME	*Bombus* spp.	Leps	XYVI
		SPPE		SPIC	Non-sphecid wasps	Non-sphecid wasps	SPIC
					Leps	*Chaulignathus* spp.	APME
					Chaulignathus spp.	XYVI	HADI
A. verticillata	All wasps	*Bombus* spp.	SPIC	SPIC	XYVI	SPPE	*Myzinum* spp.
	Bombus spp.	XYVI	*Bombus* spp.	SPPE	APME	*Chaulignathus* spp.	*Bombus* spp.
	APME	SPIC	XYVI	*Bombus* spp.	SPPE	SPIC	SPPE
	Leps	APME	SPPE	APME	Non-sphecid wasps	XYVI	SPIC
	XYVI	SPPE	APME	Leps	*Bombus* spp.	*Bombus* spp.	
				XYVI	SPIC	Non-sphecid wasps	
					Chaulignathus spp.	APME	
					Leps	*Myzinum* spp.	
						Leps	

to be tested is that the small flowers of *A. incarnata* relative to *A. syriaca* may allow more contact points/flower visit and, hence, more pollinium removals. Its vertical umbel position during insect visits versus the flexible *A. verticillata* pedicels and lax peduncles of *A. syriaca* may have a similar effect. The relatively flat-topped, upright umbels or other floral cues may also influence the high prevalence, constancy, and duration times of large lepidopterans (e.g., *Danaus, Papilio, Battus*) on *A. incarnata*. Long foraging times for non-native honeybees on *A. syriaca* relative to native pollinators further suggest that geitonogamous selfing may reduce the effectiveness of *Apis mellifera*. Similarly, the number of *A. incarnata* plants visited per bout by honeybees averaged 0.25–0.5 times that of native *Bombus, Xylocopa*, and both nymphalid and papilionid butterflies, corroborating the high potential for geitonogamy detected by Ivey et al. (2003) for *A. mellifera* relative to other pollinators.

Pollinators varied with respect to constancy, but the idiosyncratic nature of variation in constancy among plant species suggests that species-specific floral traits do not consistently induce fidelity. In contrast, different insect taxa varied significantly in their probabilities of moving conspecifically when foraging, and this behavior varied seasonally and with the plant species visited (Fig. 2C). Annual variation in insect emergence and flowering phenology (e.g., Wiggam & Ferguson, 2005) can potentially influence the relative abundance or efficiency of pollinators over the course of a season or between years. For all milkweeds, constancy was highest during peak flowering, when pollinators are primarily foraging within a species. This increases potential intraspecific pollen transfer while maximizing resource gains to insects by decreasing handling time (Slaa & Biesmeijer, 2005). In all, further research is essential to understand the dynamics of foraging variability in multispecies assemblages and its effect on pollination efficiency, e.g., the near absence of *Xylocopa virginica* on *Asclepias syriaca* likely reflects its late season emergence, but this requires more intensive study across years.

A third goal of our study was to compare several commonly used pre- and post-visit indices of pollinator effectiveness across co-occurring plant populations. We especially sought to compare major pollinators for two widely used measures: (1) pollen load on captured insects, and (2) pollen removal from anthers and deposition on stigmas after single visits of insect pollinators to virgin umbels. In both measures, non-target variables that may influence the outcome-dependent variables are not fully controlled. Vector pollen loads are potentially affected by the insect age and foraging time. The pollinium load on a captured insect is not a direct evaluation of pollinium removal or insertion and is confounded by pollinium loss due to insect grooming or other behaviors (Lynch, 1977; Morse, 1985). Similar challenges exist for single-visit data using virgin umbels. Although some investigators inspect pollinators after a floral visit (Lynch, 1977; Morse, 1985; Fishbein & Venable, 1996; Ivey et al., 2003), most researchers find it highly impractical to measure the pollen levels on an animal's body prior to floral visitation. This problem has been little discussed in the literature (Morse, 1985), yet a high prior pollen load (e.g., from an insect that has foraged all morning vs. a newly emerged insect) likely affects the probability of pollen deposition on virgin flowers. Some of the bias involved in capture data should be diminished by a large sample size or by observing pollinium removal and insertion after single visits to virgin flowers. Although we found some congruence in these measures, low visitation rates in the populations used for single-visit analyses and shifts in weather and mowing schedules on sampling dates prevented a more robust comparison of effectiveness based on capture data and single-visit removal rates.

We detected variation in pollinator fidelity, pollinia removal, and pollinator efficiency among sympatric species of *Asclepias*. Overall, pollinators were more efficient in transferring pollinia of *A. syriaca*, although they carried more pollinia of *A. incarnata*. Native and non-native bees performed similarly across taxa, although the non-native honeybee, *Apis mellifera*, tended to be inefficient in transferring pollinia even after high removal rates (Table 2). Further exploration of the correlations among pre- and post-contact measures of pollinator effectiveness as well as designing of experiments to evaluate the potential associations of lepidopterans and hymenopterans with effective pollination of *Asclepias* species that differ in floral traits would be beneficial to our understanding of this system. Overall, we know remarkably little about how generalized pollination systems affect floral divergence and about the effect of spatio-temporal variation in pollinators on sympatric populations of plants with differing floral cues or architectures.

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REVISION OF *WIELANDIA*, INCLUDING *BLOTIA* AND *PETALODISCUS* (PHYLLANTHACEAE; EUPHORBIACEAE S.L.)[1]

Petra Hoffmann[2] *and Gordon McPherson*[3]

ABSTRACT

The Western Indian Ocean genus *Wielandia* Baill. (Phyllanthaceae, Phyllanthoideae, Wielandieae) is revised, recognizing 13 species, including the new species *W. unifex* Petra Hoffm. & McPherson. As a result of recent molecular phylogenetic work and the discovery of the enigmatic *W. unifex*, all species of *Blotia* Leandri and *Petalodiscus* Baill. are subsumed under *Wielandia*. The following new combinations result: *W. bemarensis* (Leandri) Petra Hoffm. & McPherson, *W. fadenii* (Radcl.-Sm.) Petra Hoffm. & McPherson, *W. laureola* (Baill.) Petra Hoffm. & McPherson, *W. leandriana* (Petra Hoffm. & McPherson) Petra Hoffm. & McPherson, *W. mimosoides* (Baill.) Petra Hoffm. & McPherson, *W. oblongifolia* (Baill.) Petra Hoffm. & McPherson, *W. platyrachis* (Baill.) Petra Hoffm. & McPherson, and *W. tanalorum* (Leandri) Petra Hoffm. & McPherson. In addition, *W. bojeriana* (Baill.) Petra Hoffm. & McPherson, *W. danguyana* (Leandri) Petra Hoffm. & McPherson, and *W. ranavalonae* (Leandri) Petra Hoffm. & McPherson are transferred from *Savia* Willd., a genus now restricted to the Neotropics. Three species, *Petalodiscus pulchellus* (Baill.) Pax, *Savia andringitrana* Leandri, and *S. revoluta* Scott-Elliot, and four varieties, *Blotia oblongifolia* (Baill.) Leandri var. *lourelii* Leandri, *Savia andringitrana* Leandri var. *micrantha* Leandri, *S. bojeriana* Baill. var. *perrieri* Leandri, and *S. platyrachis* (Baill.) Baill. var. *microphylla* Leandri, are subsumed under other species. Characters of flower, fruit and seed, leaf venation and anatomy, as well as pollen morphology are discussed and illustrated. A key to the 13 species of *Wielandia* is presented. *Wielandia fadenii*, previously known only from Kenya, is for the first time reported from Madagascar and the Comoro Islands. Seeds of *W. danguyana*, *W. fadenii*, and *W. ranavalonae* are described and illustrated for the first time, showing a wide range of embryo morphology. Distribution maps are provided for the species not previously treated as *Blotia* or *Wielandia* s. str., and IUCN Red List categories are suggested for all taxa.

Key words: *Blotia*, Euphorbiaceae, IUCN Red List, Kenya, leaf venation, Madagascar, *Petalodiscus*, Phyllanthaceae, *Savia*, *Wielandia*.

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Wielandia Baill. as circumscribed here is a genus of 13 species in tribe Wielandieae of the medium-sized pantropical family Phyllanthaceae (ca. 2000 species). This family is one of the segregates from Euphorbiaceae s.l. (APG, 2003) and corresponds with some modifications (Wurdack et al., 2004; Kathriarachchi et al., 2005) to Euphorbiaceae subfamily Phyllanthoideae (Webster, 1994b; Radcliffe-Smith, 2001). Tribe Wielandieae as circumscribed in the classification of Phyllanthaceae (Hoffmann et al., 2006) consists of six small or monospecific genera (*Astrocasia* B. L. Rob. & Millsp., *Chascotheca* Urb., *Chorisandrachne* Airy Shaw, *Dicoelia* Benth., *Heywoodia* Sim, *Wielandia* s.l.) and has a disjunct distribution throughout the tropics. *Wielandia* s.l. is the only genus in the tribe occurring in the western Indian Ocean region. Species here united in *Wielandia* have previously been treated as members of *Blotia* Leandri, *Petalodiscus* Baill., and *Savia* Willd.

The tribe had been placed at the beginning of subfamily Phyllanthoideae in classifications of Euphorbiaceae s.l. (Webster, 1994b; Radcliffe-Smith, 2001), thereby becoming the first tribe of the family listed in these treatments. This was mainly due to the relatively complete flowers with sepals, petals, and a disc in both sexes, as well as a pistillode in staminate flowers. *Wielandia elegans* Baill. is conspicuous in Euphorbiaceae s.l. for having perfectly pentamerous flowers. Webster (1994b: 36) placed *Heywoodia* from southern Africa at the beginning of his last synoptic treatment, considering it to be "perhaps nearest in morphological characters to the hypothetical ancestor of the family." Both are members of tribe Wielandieae in the recent phylogenetic classification (Hoffmann et al., 2006).

Wielandia s.l. has not received much attention from taxonomists in recent decades other than in regional treatments (e.g., Leandri, 1958; Radcliffe-Smith, 1987). The last complete revision of the group was published in *Pflanzenreich* (Pax & Hoffmann, 1922), where *Petalodiscus* was included in *Savia*, along with species from South Africa, the West Indies, and North and South America. Further contributions to the taxonomy of this group have been made by Leandri (1957), Radcliffe-Smith (1972, 1992), and Webster (1994b).

Results of molecular phylogenetic studies show that *Savia* s.l. is triphyletic (Wurdack et al., 2004; Kathriarachchi et al., 2005) with other species now classified in tribes Bridelieae and Phyllantheae (Hoffmann et al., 2006). The constituent taxa are not congruent with the sections of Pax and Hoffmann (1922). Two of their *Savia* species (*S. ovalis* (Sond.) Pax & K. Hoffm. and *S. phyllanthoides* (Nutt.) Pax & K. Hoffm.) must be referred to *Andrachne* L. s.l. (Hoffmann, 1994; 2000) in tribe Poranthereae, where they represent distinct genera (Vorontsova & Hoffmann, 2008). The remainder are clearly divided by embryo and epidermis characters as well as breeding system into a Paleotropical group represented by *Wielandia* s.l., including *Blotia* and *Petalodiscus*, and a Neotropical group containing *Gonatogyne* Klotzsch ex Müll. Arg., *Savia* s. str. (both Bridelieae; see Hoffmann & Cordeiro, in prep.), and *Savia* sect. *Heterosavia* Urb. (Phyllantheae; see Kathriarachchi et al., 2006; Hoffmann, 2008). Previously published taxonomic revisions of Wielandieae in this series include *Blotia* (Hoffmann & McPherson, 1998) and *Wielandia* s. str. (Hoffmann, 1998).

Taxonomic History

Wielandia and *Petalodiscus* were first described by Baillon (1858), differing in their pentamerous versus trimerous ovary. On page 571, *Savia* sect. *Petalodiscus* Baill. is described, with two nomina nuda listed under it (p. 572), including *Savia* (section *Petalodiscus*) *platyrachis* (Baill.) Baill. In the atlas (plate 22) contained in the same volume, there is a drawing with analysis of *P. platyrachis* Baill., which thus constitutes a valid "descriptio generico-specifica" according to Art. 42 of the International Code of Botanical Nomenclature (Greuter et al., 2000: 67). Both the generic name *Petalodiscus* and the sectional name *Savia* sect. *Petalodiscus* were simultaneously and validly created as alternative names (Art. 34.2 of this Code). Baillon proposed two more names, *Savia pulchella* Baill. (= *Wielandia platyrachis* (Baill.) Petra Hoffm. & McPherson) and *S. mimosoides* Baill. (= *W. mimosoides* (Baill.) Petra Hoffm. & McPherson), under *Savia* sect. *Charidia* Baill. in this work, and he furthermore included section *Eusavia* Baill. (only *S. sessiliflora* (Sw.) Willd. from the West Indies, now in tribe Bridelieae) and section *Actephila* (Blume) Baill. from Asia (now in tribe Poranthereae).

Baillon validated his nomina nuda in a later publication (1861: 33–35) and placed most of these taxa unequivocally in *Savia*, distinguishing the sections *Charidia* and *Petalodiscus* mainly by a short and thick floral disc versus a long and petaloid one, respectively. Müller (1866: 228–231) excluded *Actephila* and included monotypic *Wielandia* (*W. elegans*). Bentham (1878: 208) as well as Bentham and Hooker (1880: 270) excluded *Wielandia* from *Savia* again, a view adopted by authors for more than a century (Webster, 1994b; Radcliffe-Smith, 2001). In the first edition of *Natürliche Pflanzenfamilien*, Pax (1890: 15) separated all Madagascan species of *Savia* as *Petalodiscus* and retained only the Neotropical taxa in *Savia*. In *Pflanzenreich* (Pax & Hoffmann, 1922:

181) and in the second edition of *Natürliche Pflanzenfamilien* (1931: 67), however, Pax and Hoffmann reverted to a broad circumscription of *Savia*, even including two species belonging in unrelated *Andrachne* s.l. (Hoffmann, 1994). Like Baillon, Pax and Hoffmann based their sections of *Savia* on disc morphology, and the Madagascan taxa were distributed in sections *Maschalanthus* Pax (with annular floral disc; including Baillon's section *Charidia*) and *Petalodiscus* (with free disc glands).

The inclusion of West Indian, South American, and Madagascan elements in *Savia* made this genus an example of a highly disjunct transatlantic distribution (e.g., Pax & Hoffmann, 1931: 29; Leandri, 1937: 95; Howard, 1973: 35; Borhidi, 1985: 23, 1991: 262; Webster, 1994a: 21, fig. 1). The unfortunate emphasis on disc morphology in the sectional classification added to the taxonomic confusion. Baillon himself remarked (1868: 345) that *Savia bojeriana* Baill. stood between sections *Charidia* and *Petalodiscus* in *Savia*. Leandri (1958) questioned the suitability of the disc morphology for sectional classification but used these sections nonetheless. How unnatural these sections really are becomes clear in the present revision, where two species of *Savia* section *Maschalanthus* (*S. pulchella* and *S. revoluta* Scott-Elliot) are synonymized with two species of section *Petalodiscus* (*S. platyrachis* and *S. bojeriana*, respectively).

Leandri (1957: 241, fig. 7.14) was the first author to draw attention to the remarkable seed characters of this heterogeneous group treated as *Savia*. Only concerned with the Madagascan species, he found that some species of section *Maschalanthus* had thin, plicate embryos, whereas the remainder had thick, roughly hemispherical cotyledons. This led him to describe the first group as genus *Blotia* with initially five species, three of which had been described previously in *Savia*. Leandri also described three new *Savia* species and three new varieties from Madagascar between 1934 and 1958.

The last species to be described in this group was one of the most intriguing. *Savia fadenii* Radcl.-Sm. is the only species occurring in continental Africa and was initially only known from southeastern Kenya. Radcliffe-Smith (1972: 508) created the monotypic *Savia* sect. *Afrosavia* Radcl.-Sm. to accommodate this species, and he later (1992: 679) transferred it to *Petalodiscus*. In the course of this revision, a sizeable amount of material of this species from northern and western Madagascar was found in the Paris herbarium. Why Leandri never described this distinctive species remains unknown. Recently, collections of it were made from the Comoro Islands, closing the gap in distribution.

Generic Classification

There has been a considerable amount of taxonomic confusion about the generic circumscription of the Madagascan representatives of tribe Wielandieae. Webster (1994b: 36) and Radcliffe-Smith (2001: 9) accepted *Blotia*, *Petalodiscus*, and *Wielandia* at generic rank. They erroneously placed in *Savia* s. str. a number of Madagascan species that belong to *Wielandia* s.l., but they did not specify which species they meant. Webster noted that *Blotia* may not be distinct from *Petalodiscus* and drew attention to the monoecy and exalbuminous seeds of both genera. He mentioned section *Afrosavia* under *Savia* but said that it "actually belongs to *Petalodiscus*" (1994b: 36).

In 1998, the authors of this paper published a taxonomic revision of *Blotia* (Hoffmann & McPherson, 1998), recognizing it as a separate genus based mainly on embryo morphology but supported by other morphological characters. Our subsequent discovery of the embryo of *Petalodiscus fadenii* (Radcl.-Sm.) Radcl.-Sm. (Fig. 3G, H) brought up new problems. This embryo was plicate like that of *Blotia* but had a consistently different folding pattern. Other characters of *P. fadenii*, such as the small, caducous stipules and the large (relative to flower size), deeply lobed disc agreed with other *Petalodiscus* species, and others again (such as the pubescent floral disc) were unique in tribe Wielandieae. This once more made the generic boundaries appear doubtful, to the point that we considered raising section *Afrosavia* containing only *P. fadenii* to generic rank.

A single, incomplete specimen further eroded the boundary between *Blotia* and *Petalodiscus* so that we came, somewhat reluctantly, to think that we must subsume the former as a section under the older generic name *Petalodiscus*, thereby uniting all Western Indian Ocean Wielandieae with the exception of *Wielandia elegans* (which is characterized by having a 5-merous ovary) in one genus. The specimen in question (*R. Randrianaivo & R. Bernard 556*, duplicates at K, MO, TAN, US) was collected in 2000 near Antalaha in northeastern Madagascar. It bears dehisced fruit, but no mature seeds and no flowers. There are, however, sufficient characters to identify it as a new species, and it is described below as *W. unifex* Petra Hoffm. & McPherson. It has aspects of both section *Afrosavia* and *Blotia*. It resembles section *Afrosavia* in its long and thin petioles and the long, narrow leaf apex, but the terete petioles, the glabrous, short, alternipetalously lobed disc, and the small spathulate petals recall former *Blotia* species. With regard to leaf areolation, this collection stands between *Blotia* and *Petalodiscus*. No matter what other characters future collections of this taxon bring

to light, it seemed clear that *Blotia* and *Petalodiscus* are too closely related to be treated as separate genera.

Finally, molecular phylogenetic studies on Phyllanthaceae (Wurdack et al., 2004; Samuel et al., 2005; Kathriarachchi et al., 2005) showed that *Wielandia elegans*, despite its divergent pentamerous ovary, is also embedded in *Petalodiscus* s.l. and therefore cannot be maintained as a separate monotypic genus if *Petalodiscus* is to be monophyletic. We then decided to unite the entire clade (clade F4b, fig. 3 in Kathriarachchi et al., 2005) including *Blotia* and *Petalodiscus* as *Wielandia* s.l., as circumscribed in this revision. Given the choice between *Petalodiscus* or *Wielandia* as generic name, we chose *Wielandia* because this name has been known in Madagascar throughout its botanical history. The name *Petalodiscus* was used at generic level only three times, all in global synoptic treatments. The first was by Pax (1890: 15), who changed his mind just over 30 years later (Pax & Hoffmann, 1922) to unite both Neo- and Paleotropical species in *Savia*. This view, confusing the genera now called *Savia* and *Wielandia* (sensu Hoffmann et al., 2006), has also been adopted in two more recent treatments by Webster (1994b: 36) and Radcliffe-Smith (2001: 9), who nevertheless also recognized *Petalodiscus* at generic rank. To make a clean break with this confusion and to avoid introducing a new generic name into Madagascan botanical literature, we decided to use the unequivocal name *Wielandia*, which has never been applied to any plant outside the Western Indian Ocean.

Because this generic concept evolved over several years, descriptions of *Wielandia elegans* and the species formerly known as *Blotia* are treated in separate publications (Hoffmann, 1998 and Hoffmann & McPherson, 1998, respectively). For the same reason, *W. elegans* and the newly described *W. unifex* were not included in the detailed leaf and pollen morphological analyses below (the latter is also based on an incomplete specimen). The results of the above molecular analyses also showed that some of the morphologically defined sections are not monophyletic. We therefore decided to discard the sections and to order the species alphabetically (see Appendix 1), while stressing that the five species formerly included in *Blotia* form a homogeneous, easily recognized group within *Wielandia*.

Morphology

Flowers

The flowers of *Wielandia* show great morphological variety. They are between 1 and 10 mm long and wide, depending on the species. Flowers can be terminal, axillary, and cauline in the same individual. All species are monoecious. The isomerous perianth is well differentiated into calyx and corolla. The petals are half as long to several times longer than the sepals and are yellow, orange, or red in some species. Staminate flowers are generally more delicate than pistillate flowers and tend to have shorter petals relative to the sepals. This is in contrast to other Phyllanthaceae genera, such as *Andrachne* and *Leptopus* Decne., where the petals of pistillate flowers are less developed than those of staminate flowers.

Some taxa, e.g., *Wielandia platyrachis*, have thick petals protecting the flower in bud, whereas in others, e.g., *W. fadenii* (Radcliffe-Smith) Petra Hoffm. & McPherson, the petals are membranous and completely folded into the sepals before anthesis. The petals in *W. platyrachis* (Fig. 1L–N) were incorrectly reproduced in Baillon (1858: t. 22) but noted by Leandri (1958: fig. 20.3), who unfortunately mislabeled them as sepals in the caption (cf. his description on page 119). They have a conspicuous basal thickening on the adaxial surface obscuring the very short disc. There is no indication that these thickenings function as nectaries.

Disc shape and size relative to flower size is variable, extremes being again *Wielandia platyrachis* (Fig. 1L, N), with a disc attaining ca. one seventh the total size of the flower, and the staminate flowers of *W. danguyana* (Leandri) Petra Hoffm. & McPherson (Fig. 1D), with a disc that is about half as long as the entire flower. It is ironic that the first species is the type of *Petalodiscus*, because the genus includes species with much more obvious and elaborate floral discs. The disc lobes, where present, are alternipetalous and sometimes distally emarginate or 2-lobed (Fig. 1A, D, H). Other genera of Phyllanthaceae such as *Andrachne* and *Phyllanthus* L. have alternisepalous disc glands, standing before the petals in *Andrachne*, and in the position of the petals in the apetalous genus *Phyllanthus*.

The varied disc morphology displayed by the species of *Wielandia* prompts the question whether the disc of Euphorbiaceae s.l. is derived from an intrastaminal whorl of floral organs, or whether it is of a receptacular nature. This debate was summarized by Webster (1994a: 11), with the conclusion that the available evidence favors the latter hypothesis. In fact, none of the disc glands of *Wielandia* could, on close examination, be mistaken for petals or staminodes in texture or shape. Elaborate receptacular nectaries are known from other families, e.g. Passifloraceae and Rutaceae (Weberling, 1989: 22, 195).

The androecium of *Wielandia* is isomerous with the perianth, and the stamens are alternipetalous. The

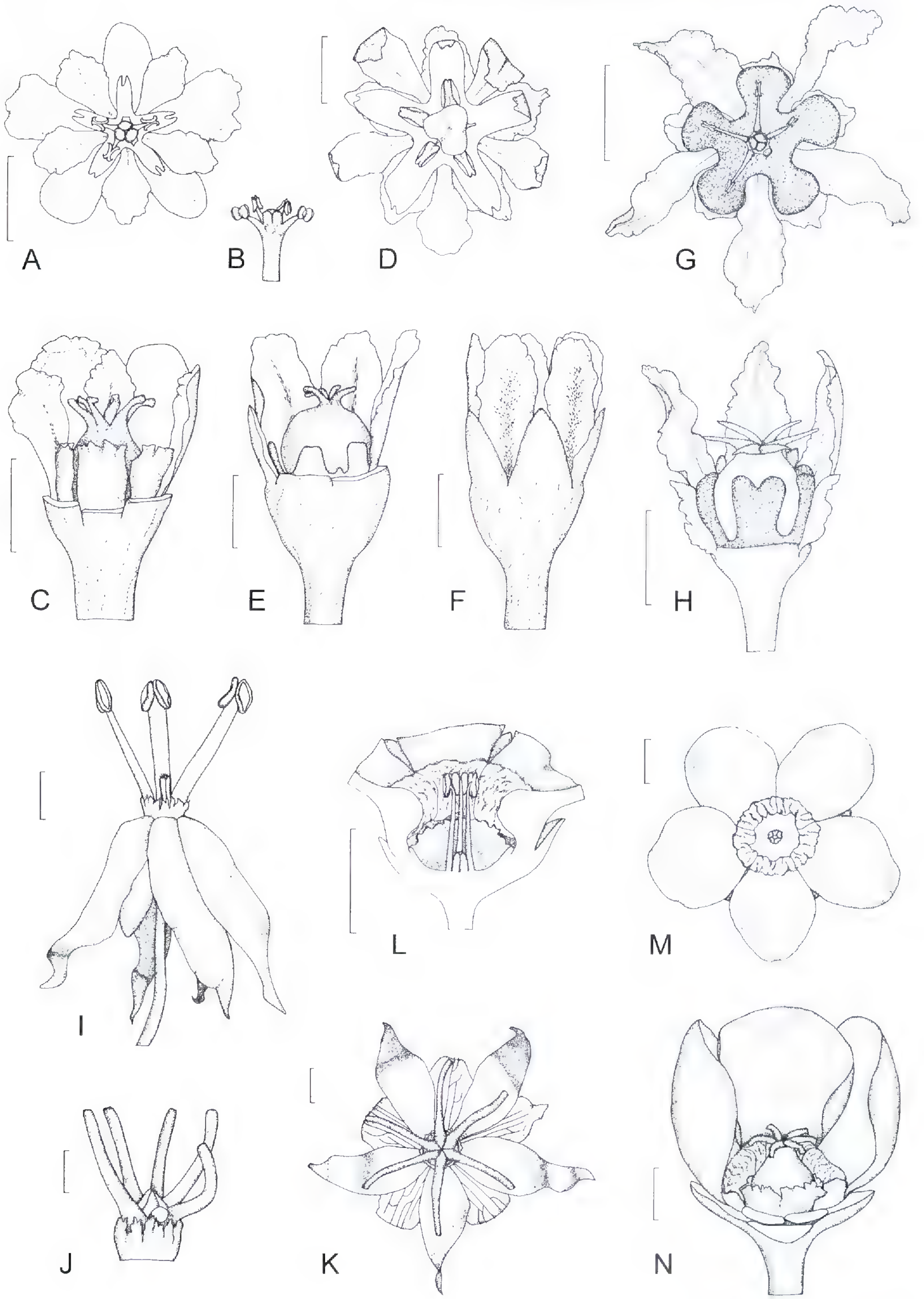

Figure 1. Flowers of *Wielandia*. A–C. *W. laureola*. —A. Staminate flower. —B. Androecium. —C. Pistillate flower, parts of perianth removed. D–F. *W. danguyana*. —D. Staminate flower. —E. Pistillate flower, parts of perianth removed. —F. Pistillate flower. G, H. *W. fadenii*. —G. Staminate flower. —H. Pistillate flower, parts of perianth removed. I–K. *W. bojeriana*. —I. Staminate flower, two stamens removed. —J. Disc and gynoecium with conical center. —K. Pistillate flower. L–N. *W. platyrachis*. —L. Staminate flowers, longitudinal section, petals cropped. —M. Staminate flower. —N. Pistillate flower, parts of perianth removed. A, B, *Schatz & Lowry 1303* (K); C, *Capuron 22083-SF* (P); D, *Gouvernement de Madagascar 59* (P); E, F, *Service Forestier 5728* (P); G, H, *Capuron et al. 2312* (P); I, *Rakotoniaina 11712-RN* (P); J, *Humbert 19759* (P); K, *Cours 710* (P); L–N, *Capuron 22908-SF* (P). Scale bars = 1 mm. Drawn by Petra Hoffmann.

Table 1. Pollen characters of *Wielandia* 1: species with plicate cotyledons. Colpi length is measured as absolute (abs.) and as a percentage of the polar axis (%). *W. fadenii: Randrianamera 89-RN* (P); *W. bemarensis: DuPuy, DuPuy & Raharilala MB372* (K); *W. mimosoides: Cours 4716* (P); *W. oblongifolia: Thourenot 1918* (K); *W. tanalorum: Humbert & Capuron 24041* (K).

	W. fadenii	*W. bemarensis*	*W. mimosoides*	*W. oblongifolia*	*W. tanalorum*
Pollen shape	spheroidal	spheroidal	spheroidal	spheroidal	spheroidal
Polar axis (µm)	19–22	17–22	20–23	21–25	22–27
Equator (µm)	17–23	17–24	22–25	20–25	22–30
Polar axis : equatorial axis	0.9–1.2	0.8–1.1	0.9–1.0	0.9–1.1	0.9–1.1
Colpi, length (abs.) (µm)	14–20	9–15	11–16	14–17	15–17
Colpi, length (%)	67–95	50–68	52–73	62–74	62–71
Exine sculpture	tectate-perforate	eureticulate	eureticulate	eureticulate	eureticulate
Width of lumina (µm)	0.5–0.7	1–2	1–2	1–2	1–2
Exine thickness at mesocolpium (µm)	1–1.5	1–2	1.5–2	1–2	1–2

filaments are fused to varying degrees in *W. elegans*, *W. laureola* (Baill.) Petra Hoffm. & McPherson, *W. ranavalonae* (Leandri) Petra Hoffm. & McPherson, and in the former *Blotia* species, and free in the remaining four species where staminate flowers are known. The anthers are free in all species. In *W. elegans* and *W. platyrachis*, the anthers are reflexed outward and pendulous, giving them a very similar appearance despite the fact that the first has connate and the latter free filaments. The anthers of *W. tanalorum* (Leandri) Petra Hoffm. & McPherson, and to a lesser extent, *W. oblongifolia* (Baill.) Petra Hoffm. & McPherson, are also reflexed, but their filaments are partly fused. *Wielandia bojeriana* (Baill.) Petra Hoffm. & McPherson is conspicuous in having long, thick filaments that are widely exserted from the reflexed perianth (Fig. 1I). This may indicate a pollination syndrome different from that of the other species, where the stamens are short and not exserted. No observations have been recorded as to the pollinators of *Wielandia*. There is a small pistillode inside the staminal whorl.

With the exception of 5-locular *Wielandia elegans*, all species have uniform 3- (rarely 4-) locular, glabrous ovaries with free, acute styles that are bifid to the base. In *W. bojeriana*, the styles are exceptionally long and terete, matching the stamens in length.

POLLEN

A number of palynological surveys including some species of *Wielandia* have been published (Punt, 1962; Köhler, 1965; Muller et al., 1989). Pollen characters vary significantly between species, and original observations from this study are summarized in Tables 1 and 2. The largest pollen grains are found in *W. bojeriana* and *W. platyrachis* (Fig. 2A, B), whereas *W. danguyana* and *W. fadenii* (Fig. 2E, F) have the smallest pollen. *Wielandia fadenii* (Fig. 2D) and *W. danguyana* differ from the remaining species of *Wielandia* (Fig. 2C; Punt, 1987: 132; Muller et al., 1989: 68, 81) in having tectate-perforate pollen grains. *Wielandia elegans*, *W. platyrachis*, and the former *Blotia* species furthermore have short colpi relative to the polar axis, whereas *W. danguyana* and *W. fadenii* have much longer colpi. *Wielandia bojeriana* and *W. laureola* stand between the two groups, resembling *W. danguyana* and *W. fadenii* in colpus length, and *W. elegans*, *W. platyrachis*, and the former *Blotia* species in exine sculpture. Pollen of *W. ranavalonae* was not studied because of the scarcity of staminate material. *Wielandia leandriana* (Petra Hoffm. & McPherson) Petra Hoffm. & McPherson was recognized after conclusion of the pollen study, but all other former *Blotia* species are palynologically similar, and by morphological inference, *W. leandriana* can be expected to resemble them closely.

FRUITS AND SEEDS

Fruit morphology in *Wielandia* is uniform with the exception of the 5-locular *W. elegans*. The typical euphorbiaceous schizocarps of all other species (Figs. 3A; 11A, D) are 3-locular, or very rarely 2- or 4-locular. Tension in the drying pericarp at fruit maturity results in explosive septicidal, loculicidal, and septifragal dehiscence into three or six valves. During dehiscence, the septa tear irregularly and often in several places (Fig. 3A). A slim, basally and apically only slightly dilated columella remains on the plant, along with the perianth and disc (Fig. 3D). Only *W. ranavalonae* has distally more strongly thickened columellae, which correspond to its unusual seed shape (Fig. 14C). *Wielandia bojeriana* and *W. laureola* appear to have a slightly fleshy mesocarp, whereas the

Table 2. Pollen characters of *Wielandia* 2: species with thick cotyledons (unknown in *W. laureola*). Colpi length is measured as absolute (abs.) and as a percentage of the polar axis (%). *W. bojeriana: Humbert 20770* (P); *W. danguyana: Service de Colonisation (Randrianasolo coll.) 59* (K); *W. laureola: Dorr 4415* (K); *W. platyrachis: Capuron 22897-SF* (P). Pollen of *W. ranavalonae* was not studied because of the scarcity of staminate material.

	W. bojeriana	*W. danguyana*	*W. laureola*	*W. platyrachis*
Pollen shape	spheroidal	spheroidal	oblate (but also spheroidal in Muller et al., 1989: 81)	spheroidal
Polar axis (µm)	25–29	15–19	20–23	22–27
Equator (µm)	25–33	15–17	23–26	24–30
Polar axis : equatorial axis	0.8–1.2	0.9–1.2	0.8–0.9	0.8–1.1
Colpi, length (abs.) (µm)	21–25	15–19	15–19	11–15
Colpi, length (%)	72–89	94–100	71–90	45–55
Exine sculpture	eureticulate	tectate-perforate	eureticulate	eureticulate
Width of lumina (µm)	2–3	0.5	1–1.5	2
Exine thickness at mesocolpium (µm)	2–3	1–1.5	2	2

outer layer of the pericarp in all other species is macroscopically undifferentiated. The dehiscence mechanism, however, does not seem to vary.

The anatropous ovules develop into more or less campylotropous seeds, whereby micropyle and hilum stay adjacent to each other. Seeds are only rarely found on herbarium material, and mature seeds of *Wielandia laureola* are still unknown. There are one or two smooth, usually sub-ovoid seeds per locule. In *W. bojeriana*, *W. elegans*, *W. platyrachis*, and the former *Blotia* species, the seeds are apiculate at the micropylar pole and have a more or less distinct, prominent, often slightly undulating ring-like structure surrounding the chalaza (Fig. 3E, F). No part of the seed is fleshy, and the prominent area resembles the rest of the seed coat in texture. This structure is here referred to as the perichalazal annulus. In *W. danguyana*, the seeds are nearly lens-shaped (Fig. 13C–E), and in *W. ranavalonae*, they are globose to ellipsoid (Figs. 3B, C; 14C), lacking an apiculum in both species. The seeds of *W. ranavalonae* also have a conspicuously large hilum in the center of the ventral face, and a micropyle that is not terminal as in all other species but positioned laterally directly above the large hilum.

Embryo characters are more varied in *Wielandia* than in any other genus of Euphorbiaceae s.l. Exalbuminous seeds like those of *Wielandia* are found in several tribes of Phyllanthaceae (Hoffmann et al., 2006) but are rare in the remaining euphorbiaceous families. Most *Wielandia* species have thick, roughly hemispherical cotyledons (Figs. 3B, C, I; 13D, E). The exact shape of the cotyledons depends on the shape of the seed. *Wielandia ranavalonae* is aberrant in having a lateral instead of a terminal radicle (Fig. 3B, C). *Wielandia fadenii* has thin, plicate cotyledons (Fig. 3G, H). These are similar to those of former *Blotia* species (Hoffmann & McPherson, 1998: fig. 3E), but the folding pattern (which is identical in all examined seeds of *Blotia*) differs consistently from that in *Wielandia fadenii*. Plicate cotyledons occur also in *Discocarpus* Klotzsch and *Lachnostylis* Turcz. (Stuppy, 1996: t. 50B and 52B, respectively), as well as in some species of *Actephila* Blume (Baillon, 1866: t. 10.6, 10.7). Each of those genera seems to have a different folding pattern of the cotyledons.

LEAF MORPHOLOGY AND VENATION

General leaf morphology in *Wielandia* is both unspecialized and variable. Length of mature leaves in *W. bojeriana*, for example, ranges from 1 to 16 cm. The leaves lack specialized trichomes, glands (apart from the glandular tip of the midvein in some species), marginal teeth, and domatia. Only two of the species have a sparse leaf indumentum, and all others are glabrous. The stipules provide a useful character to distinguish the former *Blotia* species from all other taxa. *Blotia* stipules are persistent and at least 2 mm long, whereas those of all other species are small and caducous. Another diagnostically useful character is that the midvein is more or less prominent in all species but *W. danguyana*, where it is distinctly impressed (Fig. 6I).

Leaf venation of Phyllanthaceae (as Phyllanthoideae) has been studied by Levin (1986) and, far less analytically, by Klucking (1997). The former examined

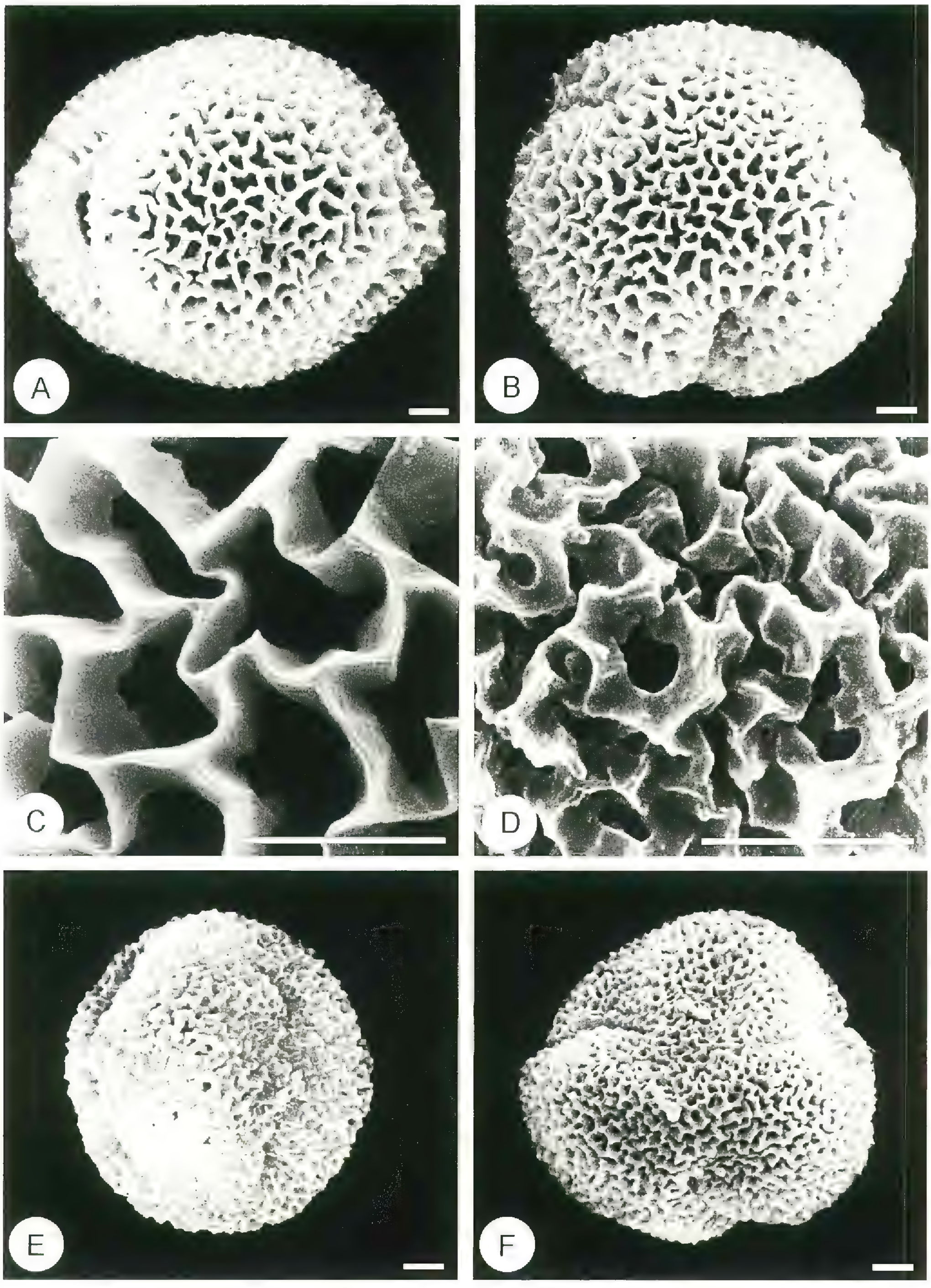

Figure 2. Pollen of *Wielandia*. A–C. *W. platyrachis*. —A. Equatorial view. —B. Polar view. —C. Detail of apocolpium. D–F. *W. fadenii*. —D. Detail of mesocolpium. —E. Equatorial view. —F. Polar view. A–C, *Capuron 22897-SF* (P); D–F, *Randrianamera 89-RN* (P). Scale bars = 2 μm.

Wielandia danguyana, *W. elegans*, and *W. oblongifolia*, whereas the latter included only *W. elegans*. The basic venation type of *Wielandia* leaves is brochidodromous, as in the majority of Phyllanthaceae (Levin, 1986: 39). Marginal ultimate venation, areolation, course of the secondary veins, and shape of intercostal fields, however, vary considerably in the genus. For terminology and categories used here, see Hickey (1979). The marginal ultimate venation of *W. bojeriana* and *W. ranavalonae*, and to a lesser degree *W. elegans* and *W.*

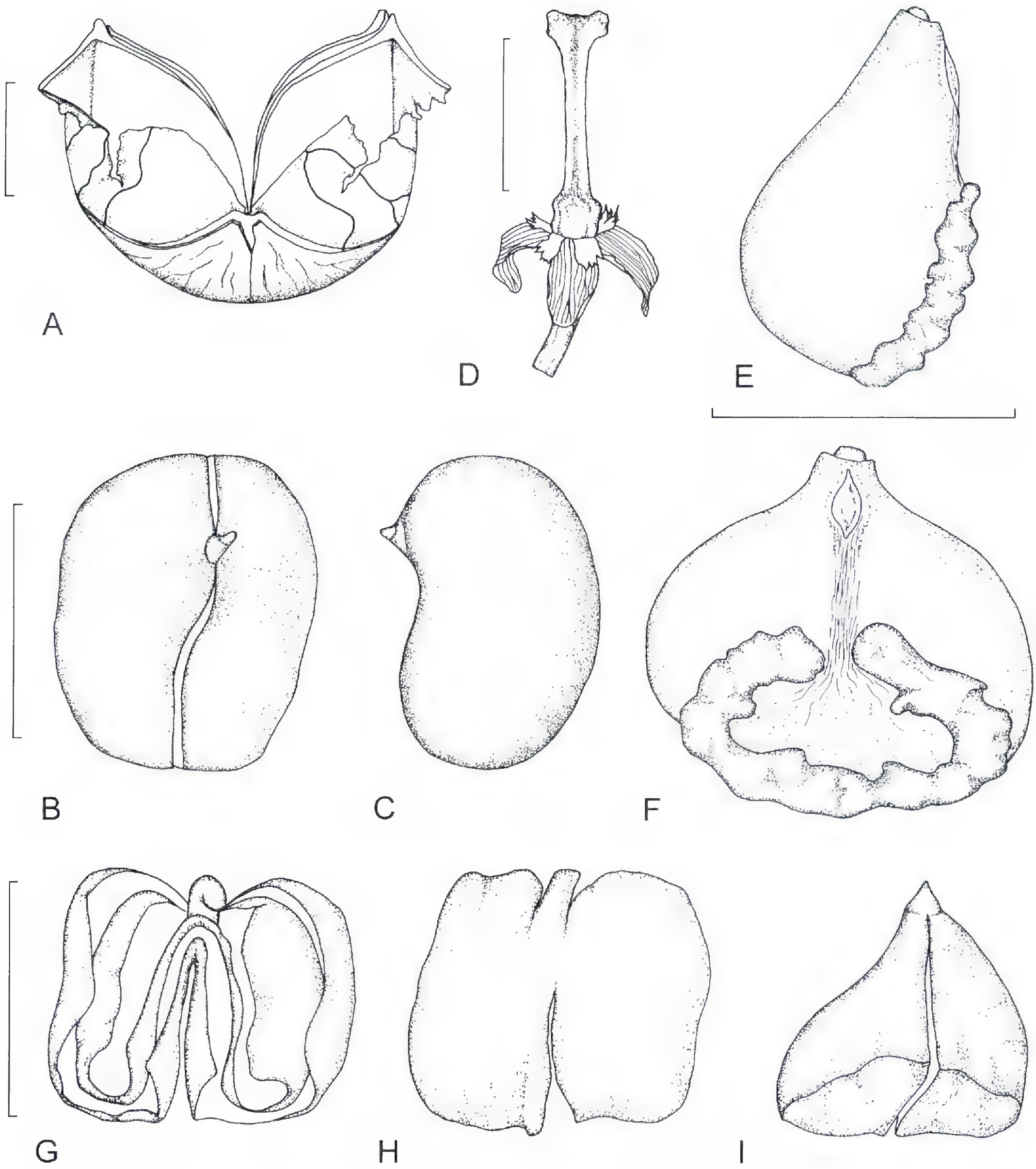

Figure 3. Fruits and seeds of *Wielandia*. A–C. *W. ranavalonae*. —A. Mericarp. —B. Embryo from 1-seeded locule in ventral view. —C. Same embryo in lateral view. D–F. *W. bojeriana*. —D. Columella with persistent perianth and disc. —E. Seed from 1-seeded locule in lateral view. —F. Same seed in ventral view. G, H. *W. fadenii*. —G. Embryo from 1-seeded locule in ventral view. —H. Same embryo in dorsal view. —I. *W. platyrachis*, embryo from 1-seeded locule in ventral view. A, *Capuron 20586-SF* (P); B, C, *Bosser 9070* (P); D, *Humbert 19759* (P); E, F, *Rabevohitra 2130* (K); G, H, *Leandri 2286* (P); I, *Service Forestier 13196* (P). Scale bars = 5 mm. Drawn by Petra Hoffmann.

platyrachis, is possibly their most interesting leaf venation character and appears to be unique at least in Phyllanthaceae. It does not seem to have been recorded before, and it warrants a new (sub)type in Hickey's (1979: 36) classification, as it is not comparable to his figures 102 or 104. We suggest that this type of marginal venation be called "exmedial." Levin also qualified the description of the marginal venation in *Wielandia* as "incomplete, veins thickened and heavily sclerified, flaring toward the margin" (1986: 40), whereas in other taxa it is simply described as "incomplete," often in conjunction with "looped." For this study, leaves from herbarium material were cleared over several weeks to months in 10% NaOH, and were briefly cleared further in 10% NaOCl. They were then stained in an alcohol solution of safranine,

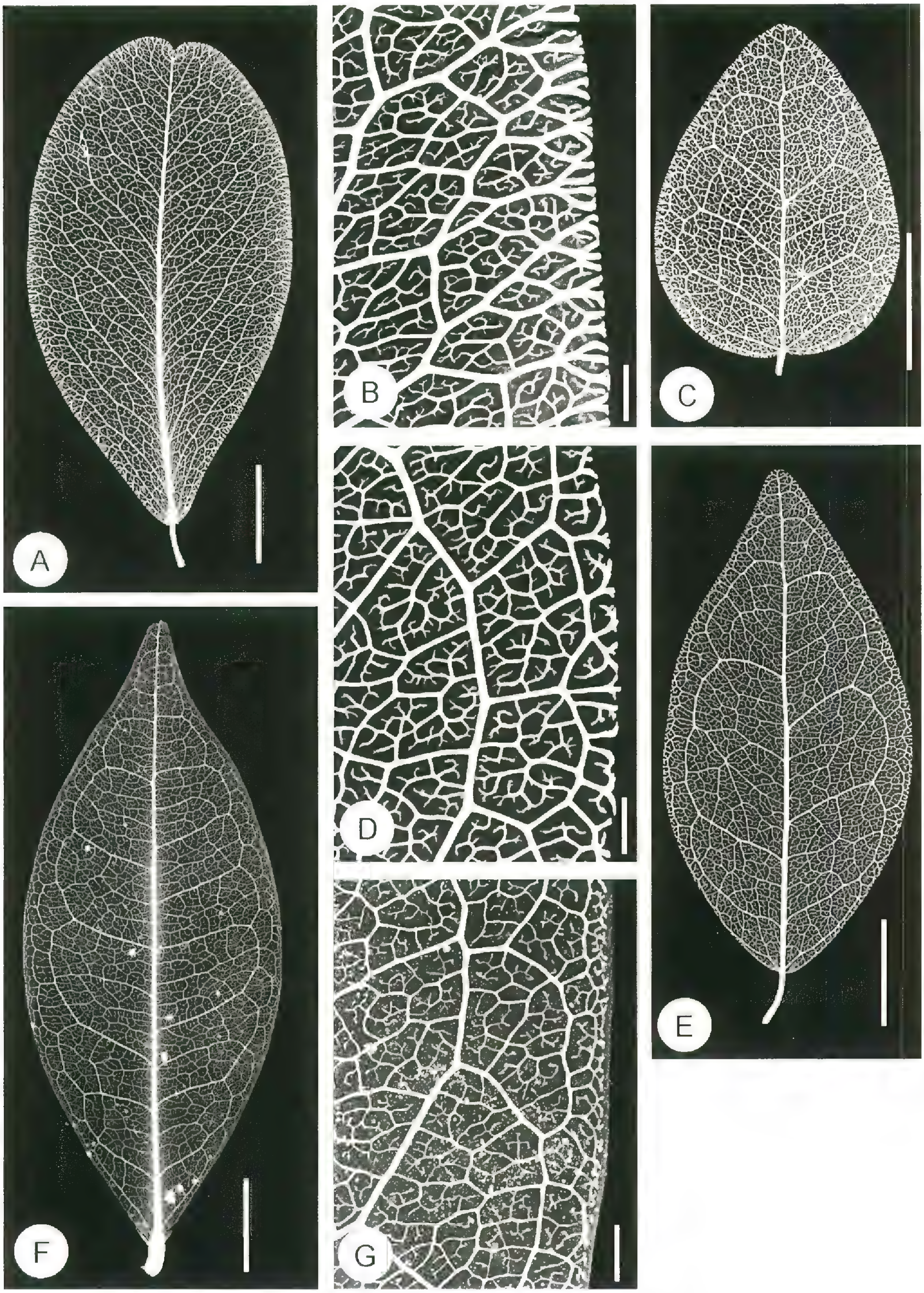

Figure 4. Leaf venation of *Wielandia* 1, from cleared leaf material. —A, B. *W. ranavalonae*. —C. *W. bojeriana*. —D, E. *W. laureola*. —F, G. *W. platyrachis*. A, B, *Bosser 9070* (P); C, *Capuron 28582-SF* (P); D, E, *Decary 6569* (US); F, G, *Capuron 23828-SF* (P). Scale bars = 1 cm in A, C, E, F; 1 mm in B, D, G.

and mounted in Canada balsam. Leaves from several specimens of each taxon were examined in this way. The photographs were taken directly from the slides onto photographic paper (without film).

The former *Blotia* species form a distinct and uniform group with respect to leaf venation that is characterized by regularity in both lower and higher order venation (Fig. 5E–H). In the remaining species

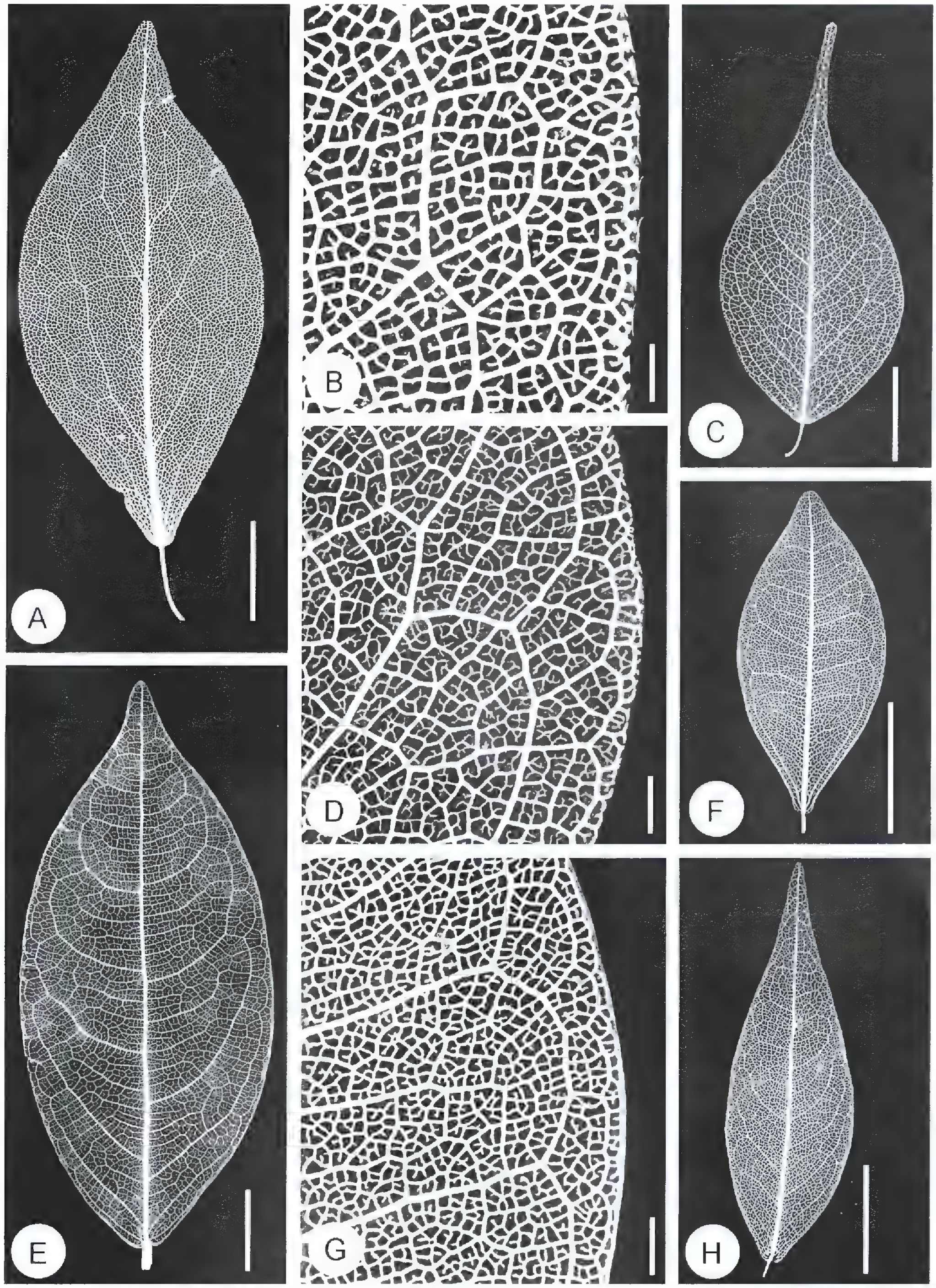

Figure 5. Leaf venation of *Wielandia* 2, from cleared leaf material. —A, B. *W. danguyana*. —C, D. *W. fadenii*. —E. *W. tanalorum*. —F, G. *W. mimosoides*. —H. *W. bemarensis*. A, B, *Capuron 11964-SF* (P); C, D, *Randrianamera 89-RN* (P); E, *Decary 5104* (K); F, G, *Thouvenot 131* (US); H, *Service Forestier 9383* (P). Scale bars = 1 cm in A, C, E, F, H; 1 mm in B, D, G.

examined in detail in this study, three groups can be distinguished: *Wielandia danguyana* (Fig. 5A, B) and *W. fadenii* (Fig. 5C, D) show irregular lower order venation, but highly organized higher order venation. The venation of *W. bojeriana* (Fig. 4C) and *W. ranavalonae* (Fig. 4A, B) generally has a low degree of organization, with highly specialized marginal ultimate venation. Finally, *W. laureola* (Fig. 4D, E)

and *W. platyrachis* (Fig. 4F, G) have very regular secondary venation contrasted with relatively unorganized higher venation.

The former *Blotia* species (Fig. 5F–H) have the most regular venation in *Wielandia*, and probably of all Phyllanthaceae. The areolation is so perfect as to resemble graph paper, with most areoles less than 0.2 mm in diameter. A similarly perfect areolation is only found in *W. danguyana*, but there the lower order venation is highly irregular (Fig. 5A, B). The former *Blotia* species have a very regular lower order venation with five to 11 secondary veins that are rounded and delimit intercostal fields of regular size and shape. The secondary veins are surrounded usually by two rows of outer loops (sometimes only one in the small-leaved *W. mimosoides* and sometimes three in *W. tanalorum*). There are long, simple (as opposed to composite), and often unbranched intersecondary veins, and tertiary veins are as a rule orthogonally reticulate, and more rarely (in the small-leaved *W. bemarensis* (Læandri) Petra Hoffm. & McPherson and *W. mimosoides*) also randomly reticulate. Marginal ultimate venation in this group represents one extreme in the genus, the other being *W. bojeriana* and *W. ranavalonae* (Fig. 4B). Only in the former *Blotia* species is the predominantly looped marginal venation sometimes partly or fully (in the large-leaved *W. oblongifolia*) fused and straightened into a fimbrial vein of the fourth order (Fig. 5G). *Wielandia oblongifolia* also lacks veinlets ending freely in the margin, which are present in varying degrees in the remaining former species of *Blotia* but never as prominent as in other *Wielandia* species. Generally speaking, differentiation and organization of leaf venation in the former genus *Blotia* decreases with decreasing leaf size, whereas it is more species-dependent in the remaining species of *Wielandia*.

Levin (1986: 43, figs. 16, 17), in his survey of foliar morphology of Phyllanthoideae, chose *Wielandia danguyana* to represent the genus *Petalodiscus* and remarked on the "unusual combination of relatively disorganized lower order venation with highly differentiated orthogonal higher order venation forming oriented, but still imperfect areoles" that he compared to *Discocarpus* (where this is less pronounced than in *W. danguyana*). *Wielandia danguyana* (Fig. 5A, B) has only four or five pairs of secondary veins, which are abruptly curved, barely extend halfway toward the leaf margin, and are surrounded by two to four rows of outer loops. The tertiary veins, on the other hand, are orthogonally reticulate, and the areoles well-developed, oriented, usually tetragonal and small to medium sized. A similarly perfect areolation is only found in the former *Blotia* species, where it is combined with a very regular lower order venation (Fig. 5E–H). The marginal ultimate venation is made up of short and thick freely ending veins with some loops. *Wielandia fadenii* (Fig. 5C, D) shows a similar but less extreme discrepancy between the different venation orders. The four to six pairs of secondary veins enclose irregular intercostal fields and are surrounded by two or three rows of outer loops. All other species, except for *W. danguyana* and *W. tanalorum*, have only one or two rows of outer loops. As in *W. danguyana*, the tertiary veins are orthogonally reticulate in *W. fadenii*, but with a slightly less organized areolation. Apart from the former *Blotia* species, *W. fadenii* shows the most pronounced looped marginal ultimate venation in the genus, with other veinlets ending freely in the margin.

The venation of *Wielandia bojeriana* (Fig. 4C) and *W. ranavalonae* (Fig. 4A, B) is of a low degree of organization and is remarkable for its conspicuously sclerified marginal ultimate venation consisting of parallel, exmedially branched free-ending veins. This can be seen on most uncleared, dried leaves in herbarium material and is an excellent diagnostic character. It is in stark contrast to leaves in former *Blotia* species and, more so, to those of West Indian *Savia* section *Heterosavia*, e.g., *S. maculata* Urb., where the marginal venation is united to form a strong fimbrial vein (P. Hoffmann, 2008). *Wielandia bojeriana* and *W. ranavalonae* have nerves thicker than average in all nerve orders. The six to 12 pairs of secondary veins are abruptly curved distally in *W. ranavalonae*, enclosing irregularly shaped intercostal fields, whereas they are rounded in *W. bojeriana*, and the intercostal fields are of a regular shape. In this, *W. ranavalonae* is more similar to *W. danguyana* and *W. fadenii*. The tertiary veins are randomly reticulate in both *W. bojeriana* and *W. ranavalonae*, and the higher venation has a tendency toward incompletely closed meshes that are randomly oriented, irregularly shaped, and medium to large.

In *Wielandia laureola* (Fig. 4D, E), a very regular lower venation with intercostal fields of nearly the same size is contrasted with unorganized higher venation. The tertiary veins of *W. laureola* are randomly to orthogonally reticulate, but the areolation is imperfect, with a tendency to incompletely closed, randomly oriented, irregularly shaped, medium to large meshes as in the two previous taxa. The marginal ultimate veins are again exmedially branching free-ending veins, but are neither parallel nor as conspicuously thickened as in *W. bojeriana* and *W. ranavalonae*. The venation of *W. platyrachis* (Fig. 4F, G) and *W. elegans* (see also Levin, 1986: 40, figs. 1, 2), finally, resembles *W. laureola* in general organization. It differs from all other species except those of the former *Blotia* species in having usually simple (as

opposed to composite), long, and unbranched intersecondary veins that can resemble the secondary veins in course and thickness. Tertiary veins and areolation are similar in degree of organization to those of *W. bojeriana* and *W. ranavalonae*, but the higher venation is very thin compared to the midvein. The only notable sclerenchymatic thickening of the higher venation is found in the ultimate veinlets, which again end freely in the leaf margin or in the areoles.

LEAF ANATOMY

Leaf anatomy in *Wielandia* is as diverse as leaf venation. The most important characters are vascularization of the petiole, sclerification of the vascular bundles in the leaf blade, course of the anticlinal walls in the epidermis, and development of stomatal ledges.

Cross sections were obtained by softening petioles and leaf fragments from herbarium material for ca. seven days in equal parts of glycerin, 96% ethanol, and distilled water (Strassburger's mixture), and sectioning at 10–25 µm with a freezing microtome. Petiolar cross sections were made at three points for each sample: at the base, in the middle, and distally just below the base of the leaf blade. To examine the epidermis, leaf fragments were macerated in Schulze's mixture ($KClO_3$ + 60% HNO_3) and 25% ammonia, and cleaned mechanically. All were stained with aqueous solutions of safranine or toluidine blue and mounted in glycerin jelly. Measurements were taken from slides prepared from different herbarium samples. Leaf surfaces were sputtered with gold and photographed (150–4000×) in SEMs at three different academic institutions in Berlin (Germany).

Basic leaf anatomy is typical for most dicots (Fig. 6J–L): bifacial, hypostomatic, with a one-layered epidermis, lacking a hypodermis, and more or less strongly sclerenchymatic or collenchymatic vascular sheaths. Trichomes are rare and, if present, are unbranched, unicellular, or uniseriate. Epicuticular wax can be copious to lacking in the same species. It regularly occurs only in *Wielandia fadenii*, where it is concentrated around the stomata (Fig. 9E). The cuticle is smooth or folded, and the epidermal cells are polygonal, irregularly shaped and oriented, and usually tabular. Anticlinal walls are undulating in *W. fadenii* (Fig. 7E), straight in all other species. Other leaf anatomical characters are summarized in Tables 3 and 4. Noteworthy is the two- to four-layered palisade of *W. platyrachis* (Fig. 6K), which consists of isodiametric cells and therefore is of about the same thickness as in the species with one or two palisade layers consisting of elongated cells.

Stomata type represents one of the most significant differences between Neotropical species of *Savia* s.l. and *Gonatogyne* on the one hand, and *Wielandia* (Fig. 7) on the other. The Neotropical taxa have paracytic stomata, whereas those in *Wielandia* are cyclocytic with three to six subsidiary cells (Fig. 7). The term cyclocytic is normally used only for stomata with four or more subsidiary cells. Stomata surrounded by three cells are called anisocytic, assuming that one subsidiary cell is smaller than the other two (Wilkinson, 1979: 100). This distinction is not justified for *Wielandia*, where there are usually three or four, and up to six subsidiary cells in the same individual, all of which can be of roughly equal size or of different sizes without following a particular pattern. Therefore, all stomata with three or more subsidiary cells are here called cyclocytic. These results are not congruent with those of Levin (1986: 33, 40–43), who indicated paracytic stomata in *W. elegans*, *W. danguyana* (as *Petalodiscus*), and *W. oblongifolia* (as *Blotia*). "Water stomata," recorded by Levin (1986: 39, 55) only for some species of *Andrachne*, were found in five of the 11 *Wielandia* species examined (e.g., *W. fadenii*, Fig. 7E).

There are considerable differences in shape and size of the stomata, *Wielandia platyrachis* and *W. ranavalonae* having much larger stomata than the remaining species (Tables 3, 4). More remarkable are the differences between the stomata of the individual species in cross section (Fig. 8). The species can be divided into three groups with regard to the development of the stomatal ledges: in *W. bojeriana* (Fig. 8C) and *W. platyrachis* (Fig. 8D), both outer and inner stomatal ledges are developed. In *W. fadenii* (Fig. 8B), *W. danguyana* (Fig. 8G), and *W. laureola* (Fig. 8F), as well as in the former *Blotia* species (Fig. 8A), only the outer stomatal ledge is developed. And finally in *W. ranavalonae* (Fig. 8E), stomatal ledges are absent. The guard cells of *W. danguyana* (Fig. 8G) and *W. laureola* (Fig. 8F), including the cuticular membrane, appear solid in light microscopy. The different shapes of the stomatal apparatus result in distinctive patterns that are confirmed in SEM (Fig. 9). These range from papillose subsidiary cells obscuring the aperture in *W. fadenii* (Figs. 8B, 9E), over outer stomatal rims in *W. bojeriana* (Figs. 8C, 9A) and *W. platyrachis* (Figs. 8D, 9B), to a mainly cuticular peristomatal rim in *W. ranavalonae* (Figs. 8E, 9F). *Wielandia danguyana* (Figs. 8G, 9D) and *W. laureola* (Figs. 8F, 9C) show a combination of the latter two states. In the former *Blotia* species, all transitions from papillose subsidiary cells (Fig. 9H) to pronounced outer stomatal rims (Fig. 9G) were observed. These characters identify *Wielandia* species by their stomata alone. They have been shown to be systematically

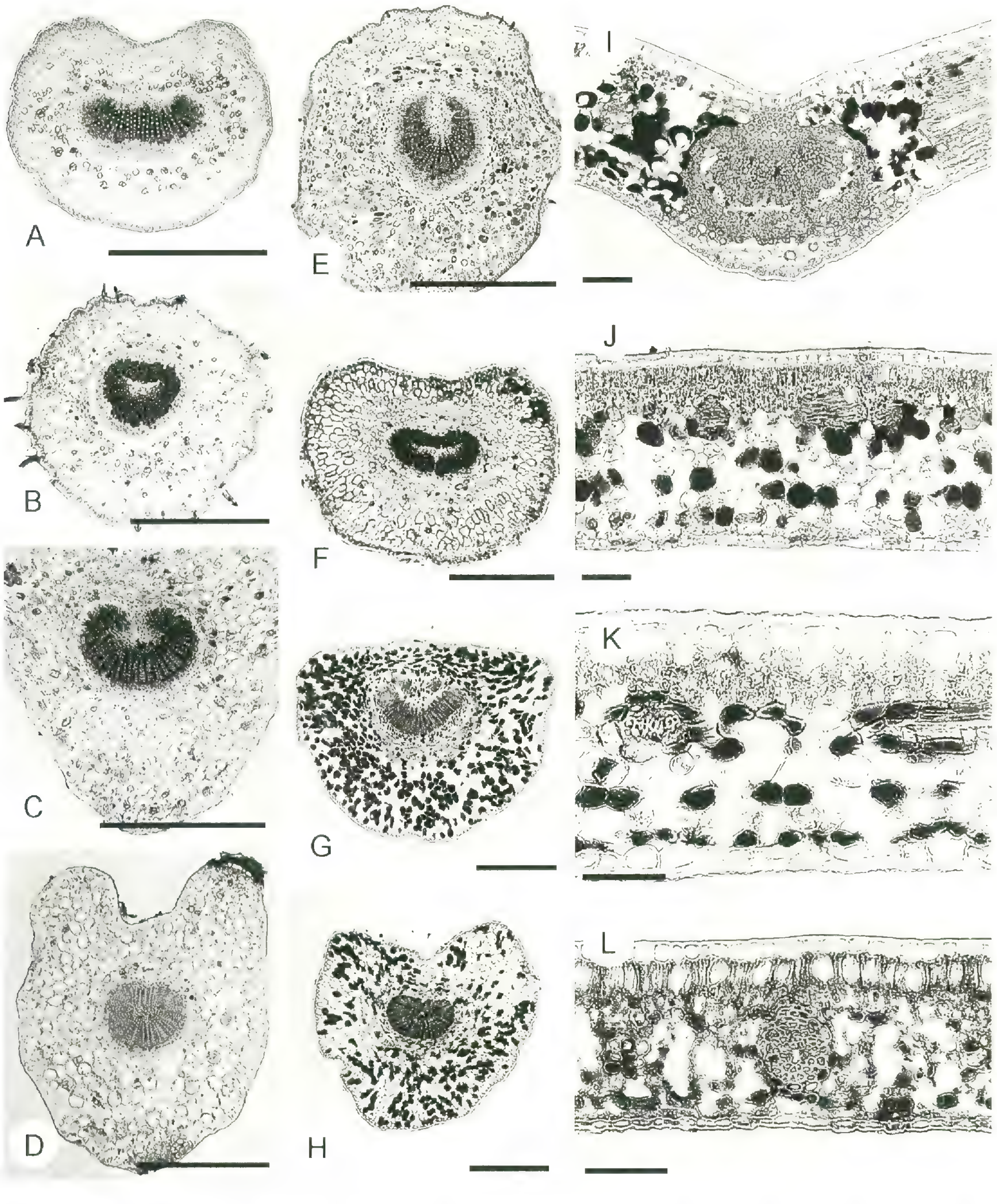

Figure 6. Leaf anatomy of *Wielandia*, petiole and leaf blade cross sections. —A, B. *fadenii*, middle of petiole. —B. *W. bojeriana*, middle of petiole. C, D. *W. platyrachis*. —C. Base of petiole. —D. Apex of petiole. —E. *W. danguyana*, base of petiole. —F. *W. ranaralonae*, middle of petiole. G, H. *W. laureola*. —G. Base of petiole. —H. Apex of petiole. —I. *W. danguyana*, midvein with subepidermal sclerenchyma. —J. *W. ranaralonae*, leaf blade. —K. *W. platyrachis*, leaf blade with isodiametric palisade cells. —L. *W. oblongifolia*, leaf blade. A, *Randrianamera 89-RV* (P); B, *Capuron 28582-SF* (P); C, D, *Capuron 23581-SF* (P); E, I, *Schlieben 8120* (G); F, J, *Capuron 20586-SF* (P); G, H, *Decary 6569* (US); K, *Capuron 11793-SF* (P); L, *Decary 6389* (P). Scale bars = 0.5 mm in A–H; 0.1 mm in I–L.

significant in other plant families at the species, sectional, and genus level (Wilkinson, 1979: 107).

The last anatomical leaf blade character to be discussed is also apparent macromorphologically. In all species, the midvein is surrounded by a sclerenchymatic sheath or cap. In 10 of the 11 species examined, these in turn are surrounded by collenchyma fibers abaxially, and collenchyma fibers and/or palisade cells adaxially. This adaxial layer of collenchyma and/or palisade cells is responsible for

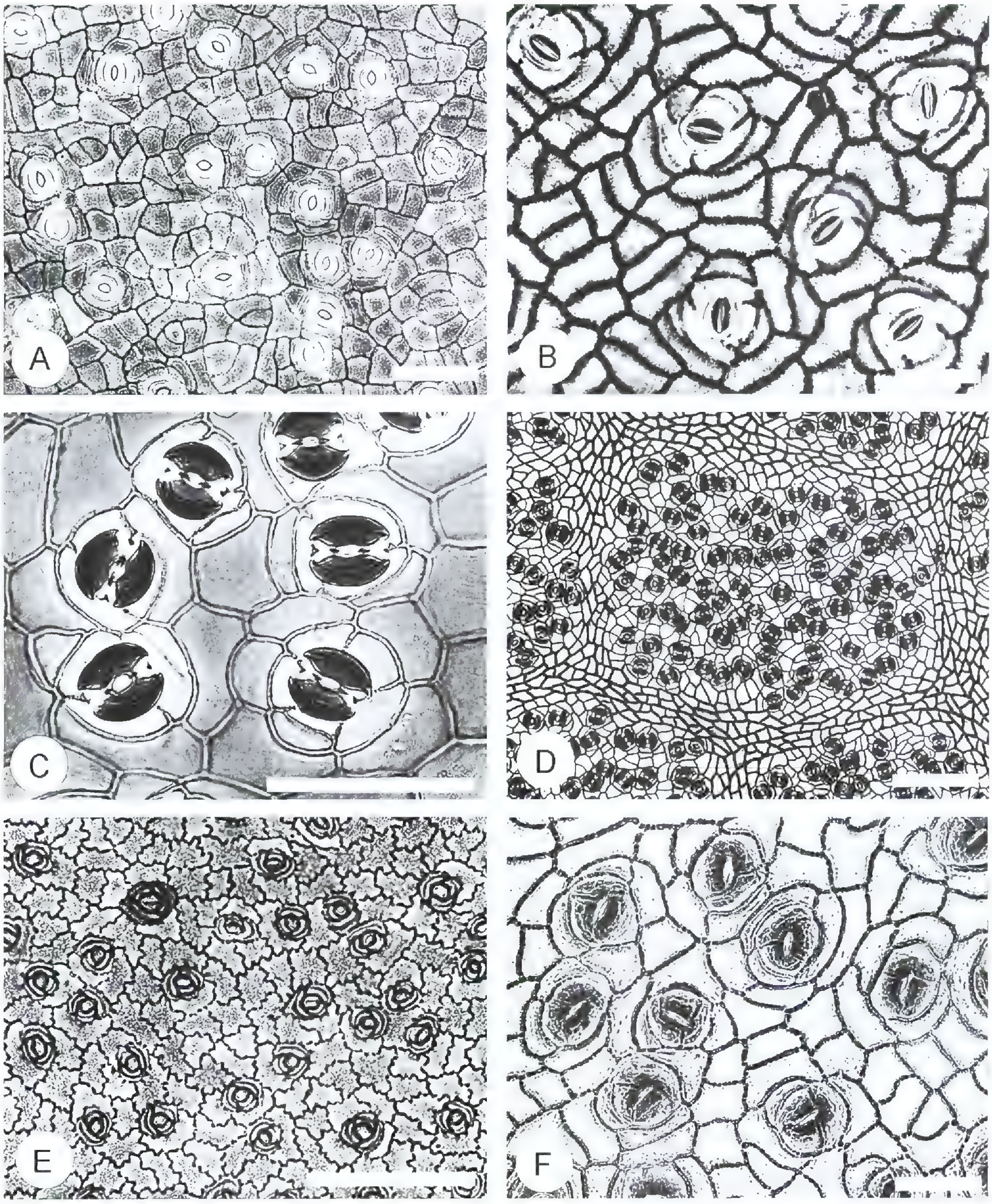

Figure 7. Abaxial leaf epidermis of *Wielandia*. —A. *W. bojeriana*. —B. *W. platyrachis*. —C. *W. laureola*. —D. *W. danguyana*, entire areole. —E. *W. fadenii*, with undulating anticlinal walls. —F. *W. ranavalonae*. A, *McWhirter & Capuron 178* (K); B, *Capuron 23555-SF* (P); C, *Decary 6569* (US); D, *Schlieben 8120* (G); E, *Randrianamera 89-RN* (P); F, *Capuron 20586-SF* (P). Scale bars = 50 μm.

the prominent appearance of the midvein in the dried leaves. In *Wielandia danguyana*, however, there is a continuous layer of sclerenchyma between the veins and the adaxial epidermis (Fig. 6I). This applies to all vein orders, with the exception of the free-ending veinlets, and results in the impressed midvein and the distinctive honeycombed appearance of the dried leaves of *W. danguyana* (Fig. 13A). The nearly perfect areolation in this species is also visible in the epidermis cells (Fig. 7D).

Finally, vascularization of the petiole is astonishingly diverse (Fig. 6A–H). This character was previously studied in the Euphorbiaceae s.l. by Dehay (1935), who did not, however, include any species of *Wielandia* in his survey. Three types can be distinguished: vascular bundle U-shaped in cross section

Table 3. Leaf anatomical characters of *Wielandia* 1: species with plicate cotyledons. *W. fadenii: Randrianamera 89-RN* (P), *Service Forestier 12398* (P); *W. bemarensis: Service Forestier 9383* (P); *W. mimosoides: Service Forestier 26166* (P), *Thouvenot 131* (US); *W. oblongifolia: Decary 6389* (P); *W. tanalorum: Decary 5104* (K).

	W. fadenii	*W. bemarensis*	*W. mimosoides*	*W. oblongifolia*	*W. tanalorum*
Epidermis, adaxial (μm)	15–20	13–20	15–20	25–40	17–20
Epidermis, abaxial (μm)	8–12	7–10	8–12	12–17	9–11
Anticlinal walls, adaxial (μm)	1–2	1	1	2–3	1–2
Anticlinal walls, abaxial (μm)	1–2	1	1	1–2	1
Periclinal walls, adaxial (μm)	4–5	5–8	5–6	6–8	5–6
Periclinal walls, abaxial (μm)	3–4	3–4	4–5	5	3–4
Stomata (μm)	17–22 × 20–25	20–25 × 18–22	20–23 × 22–25	22–25 × 22–25	16–18 × 18–20
Subsidiary cells	3(4)	3 to 5(6)	3(4)	3 to 5(6)	3 to 5(6)
Water stomata presence	+	–	–	+	–
Palisade layers	1 to 2	1 to 2	1 to 2	2	2
Ratio palisade : spongy layer	1 : 1–1.5	1 : 1.5–2	1 : 1.5–2	1 : 1.5–2	1 : 1–1.5

over the entire length of the petiole (*W. danguyana*, Fig. 6E; and *W. fadenii*, Fig. 6A); cylindrical over the entire length of the petiole (former *Blotia* species, *W. bojeriana*, Fig. 6B; *W. elegans*, and *W. ranavalonae*, Fig. 6F); or changing from basally U-shaped in cross section to distally cylindrical in cross section (*W. laureola*, Fig. 6G, H; and *W. platyrachis*, Fig. 6C, D). The cylindrical bundle is sometimes interrupted in *W. elegans* and *W. ranavalonae*, and in *W. laureola*, two additional small adaxial bundles merge with the main bundle in the middle of the petiole and contribute to the cylinder at the distal end.

Table 4. Leaf anatomical characters of *Wielandia* 2: species with thick cotyledons (unknown in *W. laureola*). *W. bojeriana: Capuron 28582-SF* (P), *McWhirter & Capuron 178* (K), *Phillipson 2919* (P); *W. danguyana: Capuron 11964-SF* (P), *Schlieben 8120* (G), *Service Forestier 2529* (P); *W. laureola: Decary 6569* (US); *W. platyrachis: Capuron 11793-SF* (P), *Capuron 23555-SF* (P), *Capuron 23828-SF* (P); *W. ranavalonae: Bosser 9070* (P), *Capuron 20586-SF* (P).

	W. bojeriana	*W. danguyana*	*W. laureola*	*W. platyrachis*	*W. ranavalonae*
Epidermis, adaxial (μm)	20–25	25–35	32–35	25–60	25–30
Epidermis, abaxial (μm)	17–20	13–18	17–20	20–40	20–25
Anticlinal walls, adaxial (μm)	2–3	3	2–3	2–3	3–4
Anticlinal walls, abaxial (μm)	1–3	1–2	1–2	2–4	2–3
Periclinal walls, adaxial (μm)	10–12	6–8	14–16	8–20	10–15
Periclinal walls, abaxial (μm)	7–10	5–6	10–12	8–25	10–15
Stomata (μm)	20–23 × 20–23	20–25 × 25–30	20–25 × 18–23	30–40 × 30–40	35–40 × 40–45
Subsidiary cells	3 to 4(5 to 6)	3 to 4(5 to 6)	3(4 to 5)	3(4)	3(4)
Water stomata presence	+	+	+	–	–
Palisade layers	2	1 to 2	2	2 to 1	2
Ratio palisade : spongy layer	1 : 3	1 : 2	1 : 2–3	1 : 2	1 : 3

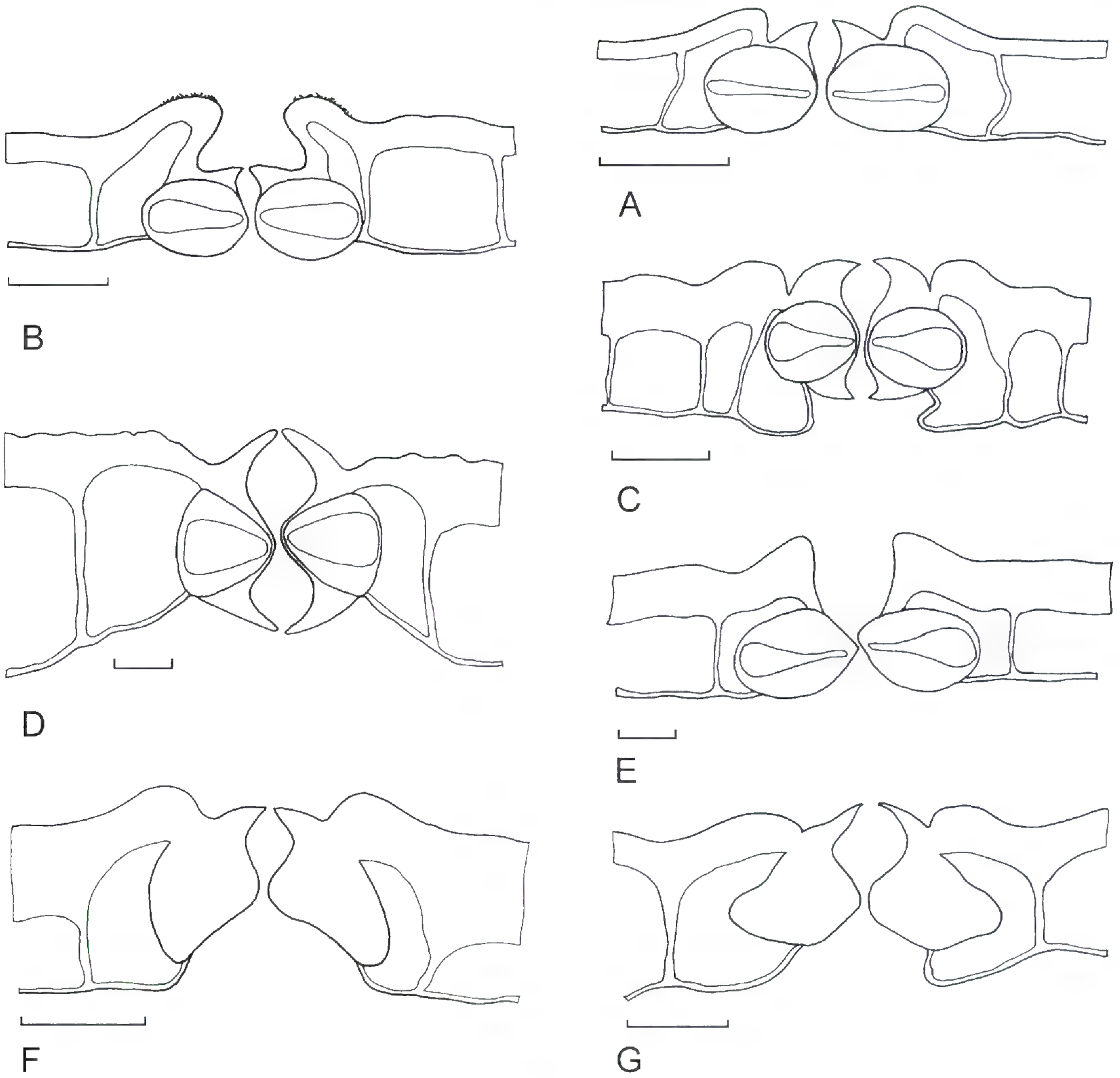

Figure 8. Stomatal apparatus of *Wielandia* in cross section. —A. *W. tanalorum*. —B. *W. fadenii*. —C. *W. bojeriana*. —D. *W. platyrachis*. —E. *W. ranavalonae*. —F. *W. laureola*. —G. *W. danguyana*. A, *Decary 5104* (K); B, *Randrianamera 89-RN* (P); C, *Capuron 28582-SF* (P); D, *Capuron 11793-SF* (P); E, *Capuron 20586-SF* (P); F, *Decary 6569* (US); G, *Schlieben 8120* (G). Scale bars = 10 µm. Drawn by Petra Hoffmann.

Biogeography and Ecology

The genus *Wielandia* occurs mainly in Madagascar. Ten of the 13 species are endemic to the island, whereas *W. ranavalonae* also occurs in the Comoro Islands, *W. elegans* also occurs in the Comoro Islands and in the Seychelles, and *W. fadenii* is found in Madagascar, the Comoro Islands, and southeastern Kenya. The latter distribution pattern is also found in other Euphorbiaceae s.l. (A. Radcliffe-Smith, pers. comm.), e.g., in *Aristogeitonia* Prain (Picrodendraceae), which has four species in Madagascar, two in southeast Kenya and northeast Tanzania, and one in Angola (Radcliffe-Smith, 2001).

In Madagascar, the genus is most commonly found in eastern wet forest but also occurs in the subhumid and montane bioclimatic zones (Cornet, 1974). The only species found in the western dry and subarid zones are *Wielandia fadenii* (Fig. 12) and, to a lesser extent, *W. bemarensis* (Hoffmann & McPherson, 1998). The collecting localities of *W. bojeriana* (Fig. 10) and *W. ranavalonae* (Fig. 11) in the predominantly subarid southwest are situated in pockets of subhumid bioclimate with deciduous but not overly dry forest (Sakaraha, Zombitsy, and the Fiherenana Valley).

Ten species are widely distributed in Madagascar, whereas the remaining three have very small distribution areas. *Wielandia elegans* (Hoffmann, 1998) is scattered across the island. *Wielandia bojeriana* (Fig. 10), and *W. mimosoides* (Hoffmann & McPherson, 1998: fig. 4B) seem to occur evenly

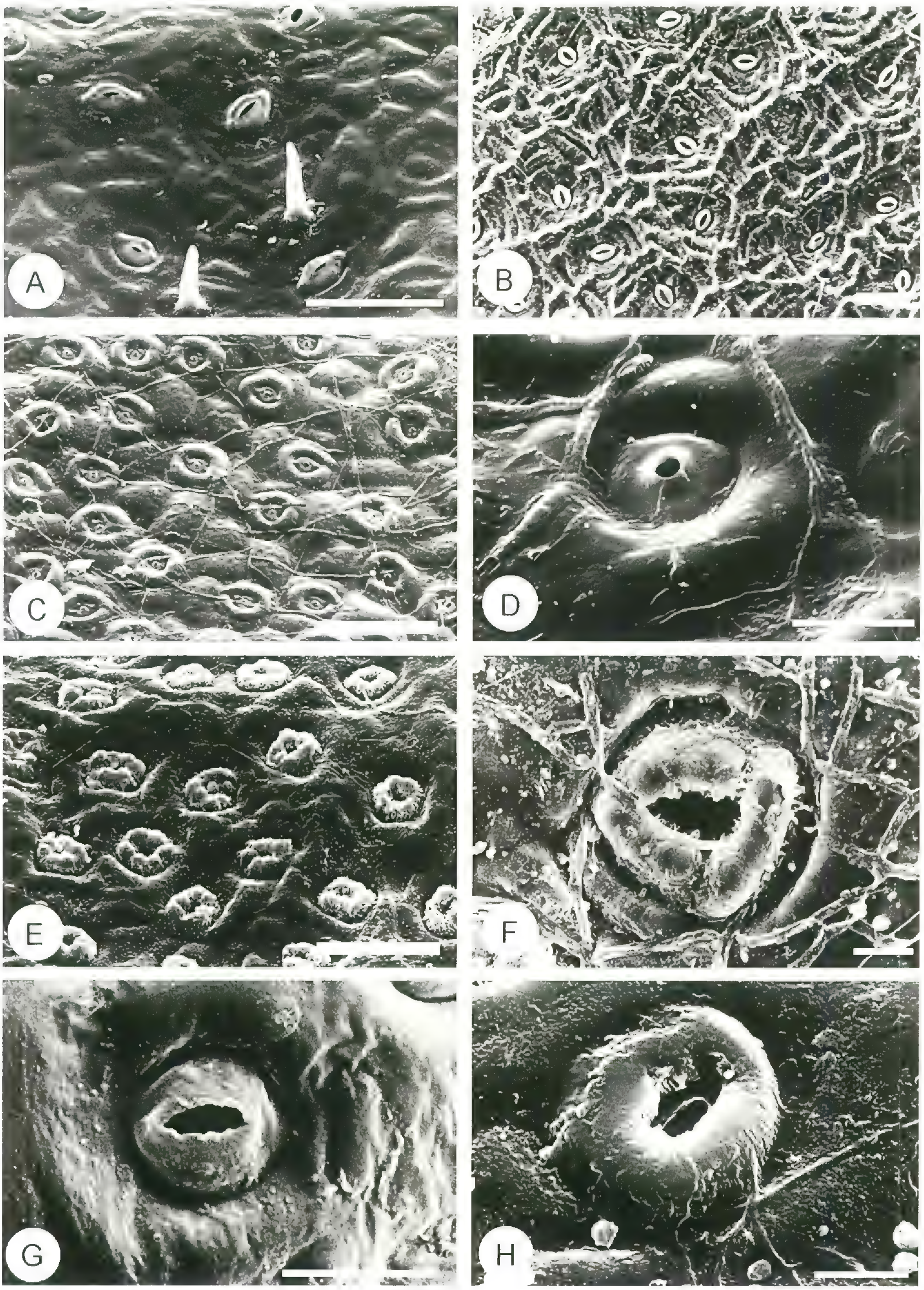

Figure 9. Abaxial leaf surface of *Wielandia*. —A. *W. bojeriana*, with trichomes. —B. *W. platyrachis*, with cuticular folds. —C. *W. laureola*. —D. *W. danguyana*. —E. *W. fadenii*, epicuticular wax concentrated around stomata. —F. *W. ranavalonae*. —G. *W. mimosoides*. —H. *W. oblongifolia*. A, *Phillipson 2919* (P); B, *Capuron 23555-SF* (P); C, *Decary 6569* (US); D, *Service Forestier 2529* (P); E, *Service Forestier 12398* (P); F, *Capuron 20586-SF* (P); G, *Service Forestier 26166* (P); H, *Decary 6389* (P). Scale bars = 50 µm in A–C, E; 10 µm in D, F–H.

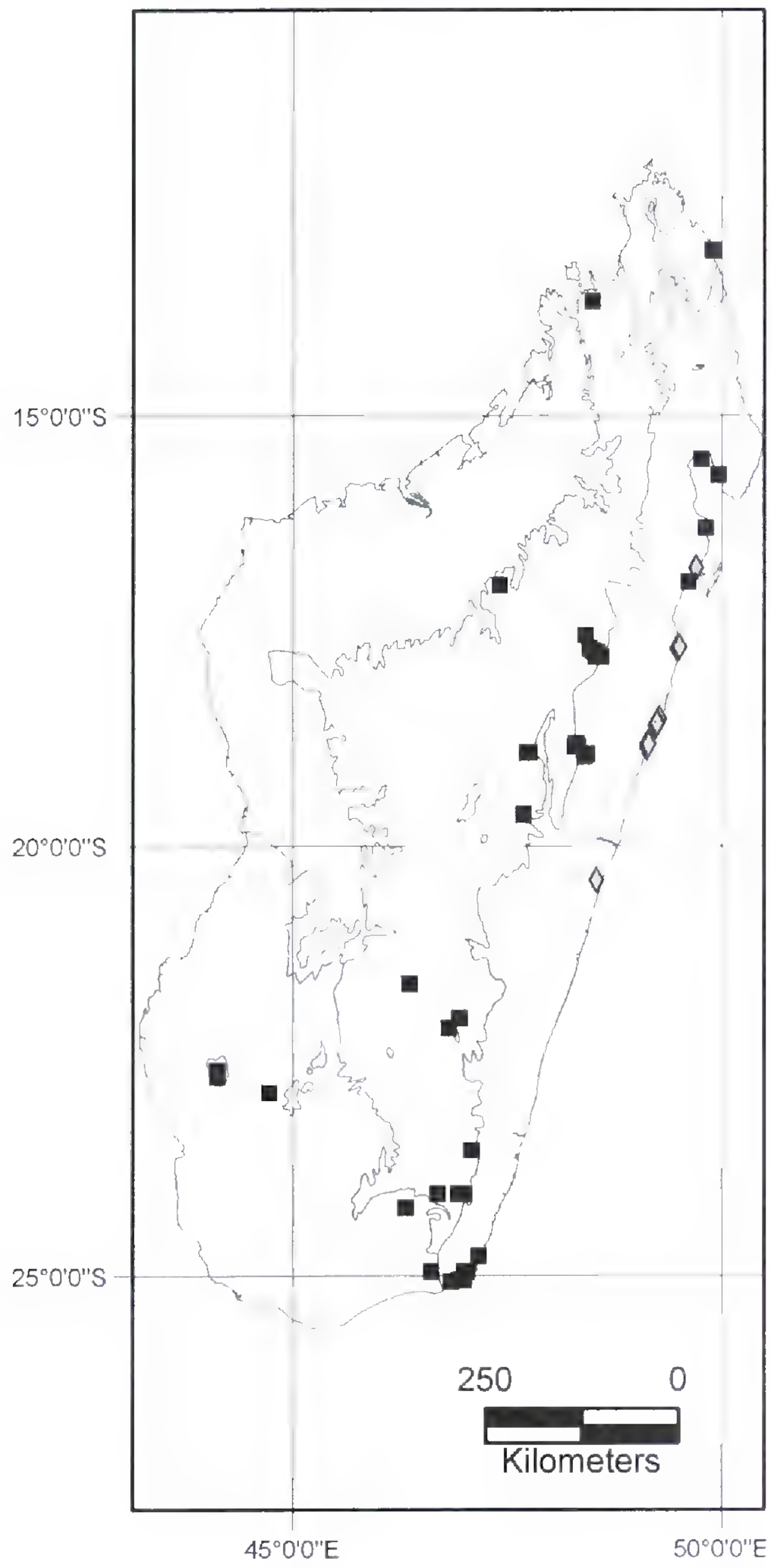

Figure 10. Distribution of *Wielandia bojeriana* (black squares) and *W. laureola* (gray diamonds with black outline).

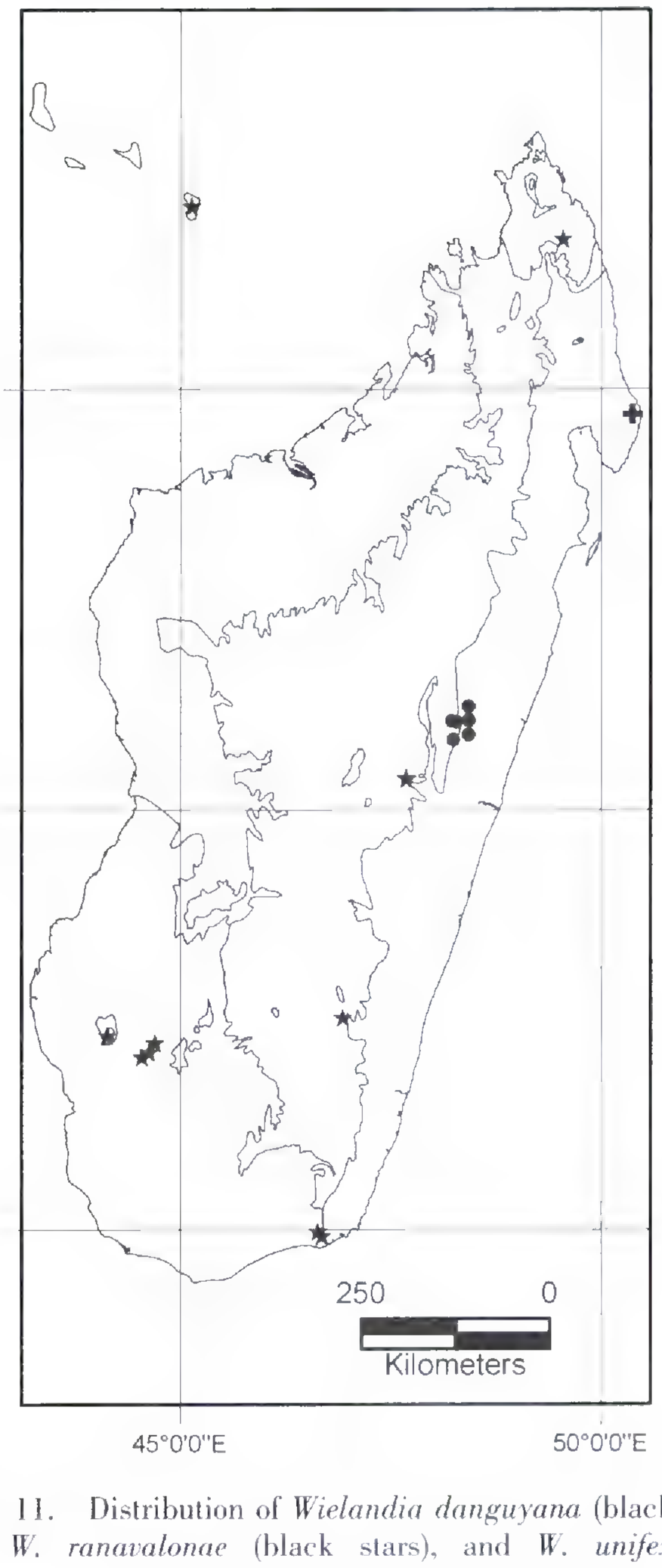

Figure 11. Distribution of *Wielandia danguyana* (black circles), *W. ranavalonae* (black stars), and *W. unifex* (black cross).

throughout the perhumid to subhumid and montane zone but have not been collected north of the Masoala Peninsula. Another widely distributed species, *W. platyrachis* (Fig. 12), is principally restricted to the perhumid zone along the east coast. The two distribution areas of *W. platyrachis* in the northwest represent pockets of humid forest, such as the Montagne d'Ambre. A similarly eastern, but less extensive distribution is shown by *W. leandriana* (Hoffmann & McPherson, 1998: fig. 4A). *Wielandia oblongifolia* and *W. tanalorum* (Hoffmann & McPherson, 1998: fig. 4C–D) extend from the humid zone to the transition between humid and subhumid zones. Information on the distribution of *W. ranavalonae* (Fig. 11) is sketchy due to the scarcity of collections, but the species seems to prefer deciduous forest in Madagascar, whereas in Mayotte it is recorded from humid forest.

Only two species prefer dry conditions. One is *Wielandia fadenii* (Fig. 12), which mainly occurs in the limestone karst areas (tsingy) of the west and the limestone massif of Ankarana in the north. An exception in Figure 12 is the morphologically divergent southernmost collection of *W. fadenii* from Farafangana on the humid east coast, which might represent a separate subspecies. The other species with a preference for dry habitats is *W. bemarensis* (Hoffmann & McPherson, 1998: fig. 4A), found mainly in the dry regions of northwestern Madagascar, with some specimens in the transition from the dry to the subhumid zone.

Wielandia danguyana and *W. laureola* are both narrow endemics inhabiting small areas in the eastern perhumid bioclimatic zone. *Wielandia danguyana* (Fig. 11) has

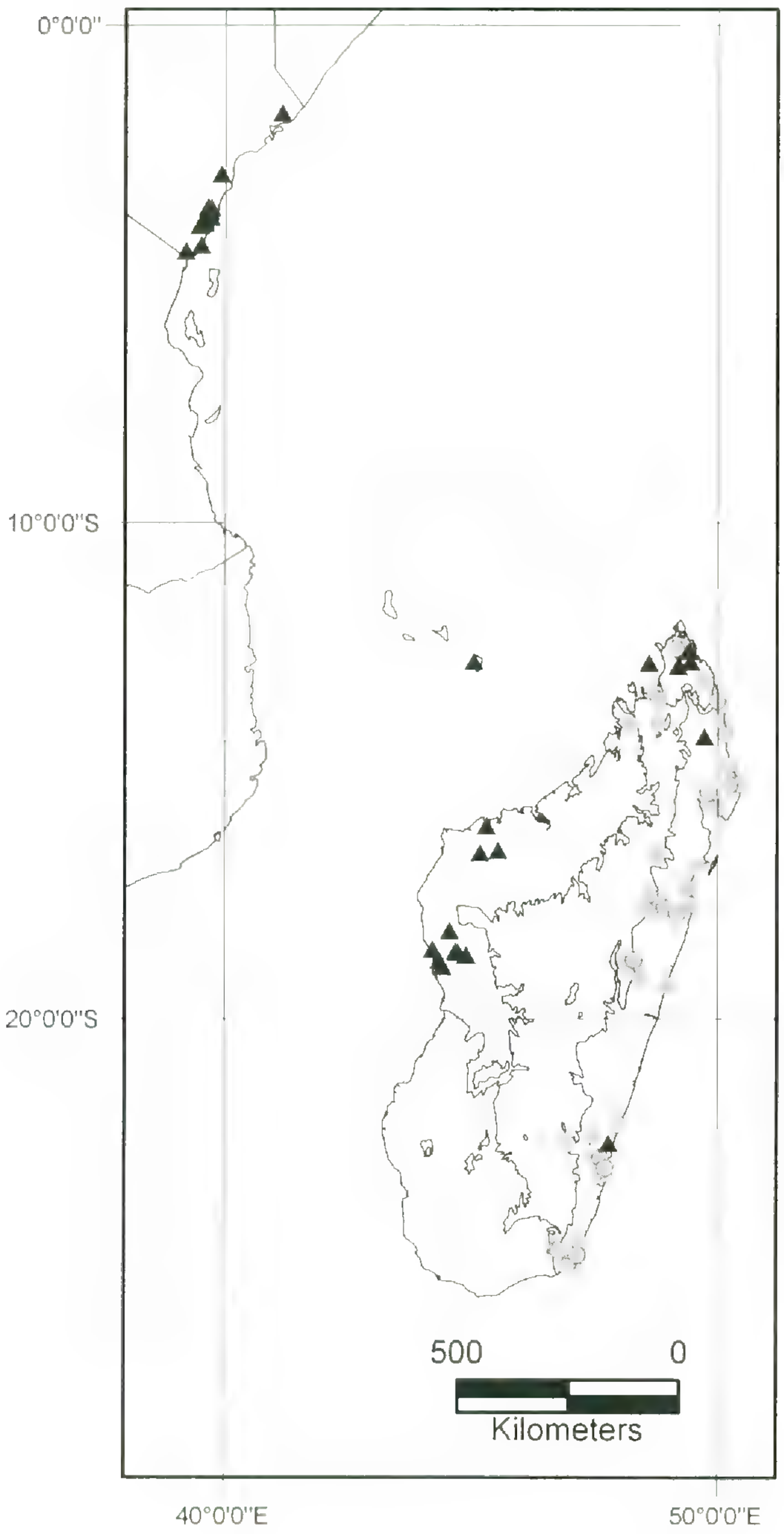

Figure 12. Distribution of *Wielandia fadenii* (black triangles) and *W. platyrachis* (gray circles).

been found only around Andasibe (Périnet), whereas *W. laureola* (Fig. 10) is restricted to the littoral white sand forest between Pointe à Larrée and Nosy-Varika. *Wielandia unifex* (Fig. 11) is only known from one collection from Antalaha in northeastern Madagascar.

Conservation

Most of Madagascar's primary vegetation has been destroyed since humans arrived on the island no more than 2000 years ago. It is estimated that only 19% of the primary native vegetation remains today, and this percentage is steadily decreasing (see www.vegmad.org). Continuing slash-and-burn agriculture (tavy), combined with land clearing for cattle grazing, pose the gravest danger for biodiversity in Madagascar. With this outlook, only species that thrive in disturbed habitats and secondary vegetation can be classified as Least Concern (LC) on the IUCN rating scale (IUCN, 2001). There is no evidence that this is the case for any species of *Wielandia*.

The 10 species *Wielandia bemarensis*, *W. bojeriana*, *W. elegans*, *W. fadenii*, *W. leandriana*, *W. mimosoides*, *W. oblongifolia*, *W. platyrachis*, *W. ranavalonae*, and *W. tanalorum* seem to be reasonably common within a distribution area that is large enough to support a genetically diverse population. All 10 are here assigned the category Near Threatened (NT) because of the threat to all natural habitats in Madagascar, despite serious efforts of the government to establish and maintain protected areas. *Wielandia fadenii* and *W. ranavalonae* also occur outside Madagascar, lessening concern for their survival despite the fact that they are ecologically more restricted and less frequently collected than the more common species.

There is, however, great concern for *Wielandia danguyana* and *W. laureola* because of their narrow distribution, and in the latter species, ecological preference for the fragile and unprotected white sand littoral forest. They are both here rated Endangered: EN B1ab(iii). *Wielandia unifex* is Data Deficient (DD), as it is only known from the type collection. For more details on the conservation status, see the individual species accounts.

Taxonomic Treatment

Wielandia Baill., Étude Euphorb.: 568, t. 22. 1858. *Savia* Willd. sect. *Wielandia* (Baill.) Müll. Arg., Linnaea 32: 78 (1863). TYPE: *Wielandia elegans* Baill., Étude Euphorb., Atlas: 40, t. 22, figs. 6–10. 1858.

Petalodiscus Baill., Étude Euphorb., Atlas: 40, t. 22. 1858, nom. altern. Syn. nov. *Savia* Willd. section *Petalodiscus* Baill., nom. altern., Étude Euphorb.: 571. 1858. *Andrachne* L. section *Petalodiscus* (Baill.) Kuntze in T. Post & Kuntze, Lex. Gen. Phan.: 28. 1903, [as "*Andrachna*"]. TYPE: *Petalodiscus platyrachis* Baill.

Blotia Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 240. 1957. Syn. nov. TYPE: *Blotia oblongifolia* (Baill.) Leandri [= *Savia oblongifolia* Baill.].

Monoecious trees or shrubs without or with weakly developed, simple indumentum; young branches sometimes flattened. *Stipules* caducous or persistent, sometimes peltate; *petioles* adaxially ± distinctly channeled or terete; vascular bundles U-shaped in cross section or cylindrical throughout, or basally U-shaped and cylindrical from the middle; *leaves* alternate, simple, eglandular; leaf blades symmetrical, entire, pinnately veined, ovate, elliptic, oblong, nearly orbicular or rarely obovate, apically acuminate to retuse, basally truncate to acute, sometimes auricu-

late; anticlinal walls of leaf epidermis straight (undulating in *W. fadenii*); stomata cyclocytic with 3 to 6 subsidiary cells. *Inflorescences* axillary or cauline; flowers directly in the leaf axils, or on unbranched or branched, usually leafless axes; staminate and pistillate flowers in the same or in separate fascicles. *Flowers* 1–10 × 1–10 mm; *pedicels* 1–30 mm long, inarticulate; *sepals* (4 or)5(or 6), quincuncial in 5-merous flowers; *petals* (4 or)5(or 6), half as long to 3× as long as sepals; *disc* extrastaminal, glabrous or rarely pubescent, of discrete alternipetalous lobes, or alternipetalously deeply lobed or crenate, or annular, or lacerated; as long as or much shorter than the sepals, sometimes of different structure in staminate and pistillate flowers; *stamens* alternipetalous; filaments free or partly fused; anthers introrse, dehiscing longitudinally, pistillode 3-fid (5-fid in *W. elegans*); *ovary* 3(to 5)-locular, locules biovulate; styles 3 (rarely 4 or 5), free, 2-fid to the base; stigmas acute to slightly capitate. *Fruits* solitary schizocarps, subglobose to globose, 3(to 5)-lobed; dehiscence septicidal, loculicidal, and septifragal; dehiscence of septae irregular; fruiting pedicels 3–50 mm long; columella 5–10× longer than the narrowest width, acutely 3(to 5)-angled, base and apex ± thickened; disc and perianth usually persistent in the fruiting stage; *seeds* 1 or 2 per locule, ecarunculate, usually apiculate and with ± distinct perichalazal annulus, globose, ovoid or ellipsoid (1-seeded locules) or nearly triangular in cross section (2-seeded locules); endosperm in mature seeds absent but for a thin membrane; embryo either with hemispherical to hemi-ovoid cotyledons and the radicle hardly longer than wide or with thin and folded cotyledons and the radicle ca. 3× as long as wide.

The genus contains 13 species; it is distributed in Madagascar, the Seychelles, Comoro Islands, and southeastern Kenya.

A complete list of specimens studied for each species is available via the Tropicos website (http://mobot.mobot.org/W3T/Search/vast.html) by searching for the species name and then clicking on "Specimen lists and maps." By clicking on any specimen in the resulting list, one can confirm that it was determined by us.

Key to the Species of *Wielandia*

1. Midvein adaxially distinctly impressed; other venation conspicuously reticulate when dry, lacking distinct secondary veins (Fig. 5A) . . . . . 3. *W. danguyana*
1′. Midvein adaxially ± prominent; other venation not conspicuously reticulate when dry, secondary veins differentiated . . 2
2. Higher leaf venation very finely areolate, resembling graph paper, most areoles less than 0.2 mm in diameter (Fig. 5G); stipules persistent, at least 2 mm long; young twigs terete, often pubescent or puberulent; cotyledons (where known) thin, folded several times (former *Blotia* species plus *W. unifex*) 3
2′. Higher leaf venation with medium-sized to large areoles, not resembling graph paper, most areoles more than 0.2 mm in diameter; stipules caducous, or persistent and no longer than 2 mm; young twigs flattened or terete, glabrous (except in *W. bojeriana*, which has maroon petals, elongate styles, and both axillary and cauline inflorescences); cotyledons hemispherical to hemi-ovoid, or folded 8
3. Sepals thickened and usually pubescent at tips; leaf blades (2.5–)4–9 cm long; petioles not channeled adaxially; flowers 3–6 mm long . . . . . 12. *W. tanalorum*
3′. Sepals undifferentiated apically, glabrous; leaves and petioles various; flowers 1.5–3 mm long 4
4. Petioles 3–10 mm long; twigs glabrous 5
4′. Petioles 1–3 mm long; twigs often pubescent 6
5. Petioles adaxially channeled, 1–1.5 mm wide; fruits 10–13 mm long . . . . . 9. *W. oblongifolia*
5′. Petioles terete, 0.5–0.8 mm wide; fruits ca. 9 mm long . . . . . 13. *W. unifex*
6. Petals (1.5–)2–3 mm long, usually longer than the sepals at anthesis, clawed only at the base; leaf blades auriculate, ovate to elliptic; western and northern Madagascar . . . . . 1. *W. bemarensis*
6′. Petals 1–1.5 mm long, as long as or shorter than the sepals at anthesis, clawed for about half the length; leaf blades auriculate or not, elliptic; eastern and central Madagascar 7
7. Leaf blades auriculate, 1.5–3(–4) cm long; pedicels less than 15 mm long; petals 1 mm long . . . . 8. *W. mimosoides*
7′. Leaf blades not or scarcely auriculate, 2–6(–8) cm long; pedicels 10–20 mm long; petals 1–1.5 mm long . . . . . 7. *W. leandriana*
8. Leaf blades long-acuminate, the tip 10–20 mm long, the sides subparallel for over half their length 9
8′. Leaf blades not or only short-acuminate, the acuminate portion very rarely over 10 mm long and then not parallel-sided 10
9. Petiole channeled; petals 1.5–2 mm long, about twice as long as the sepals (except for one specimen from SE Madagascar; see note); disc pubescent . . . . . 5. *W. fadenii*
9′. Petiole terete; petals ca. 1 mm long, shorter than the sepals; disc glabrous . . . . . 13. *W. unifex*
10. Base of petals thickened (Fig. 1L–N); young twigs and inflorescence axis always flattened or 2-winged . . . . . 10. *W. platyrachis*

10′. Base of petals not thickened; young twigs terete or flattened 11
11. Ovary and fruit (4–)5(–6)-locular; pistillode 5-lobed; flowers 4–10 × 5–10 mm; perianth parts not reflexed . . . . . 4. *W. elegans*
11′. Ovary and fruit 3-locular; pistillode 3-lobed; flowers 1–10 × 2–8 mm; perianth parts reflexed or not 12
12. Flowers 1–2 mm long, borne on ± leafless axes; leaves acute to acuminate, discolorous, with inconspicous marginal ultimate venation . . . . . 6. *W. laureola*
12′. Flowers 3–10 mm long, borne directly in the axils of regular leaves; leaves acute (very rarely acuminate) to retuse, concolorous, with usually conspicuously exmedial and parallel marginal ultimate venation (Fig. 4A–C) 13
13. Leaves ovate to nearly orbicular, acute (very rarely acuminate) to rounded; stylar branches terete, about 10× as long as wide, stigmas truncate; filaments free, ca. 0.5 mm thick, connective deltoid; columella of fruit ca. 7 mm long, up to 2 mm wide at apex; sepals of mature flowers usually reflexed; disc membranous, irregularly crenulate to deeply lacerate . . . . . 2. *W. bojeriana*
13′. Leaves obovate to nearly orbicular, retuse to rounded; stylar branches tapering, about as long as wide, stigmas acute; filaments fused for half of their length, ca. 0.2 mm thick, thecae parallel; columella of fruit 8–12 mm long, 2–3 mm wide at apex, clavate; sepals of mature flowers erect; disc fleshy, 5-lobed . . . . . 11. *W. ranavalonae*

1. **Wielandia bemarensis** (Leandri) Petra Hoffm. & McPherson, comb. nov. Basionym: *Savia bemarensis* Leandri, Bull. Soc. Bot. France 81: 588. 1934. *Blotia bemarensis* (Leandri) Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 242. 1957. Syn. nov. TYPE: Madagascar. Tsingy du Bemaraha (9e Réserve), Ambohivazimba, 150–200 m, *J. Leandri 938* (holotype, P!).

Blotia ankaranae Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 242. 1957. TYPE: Madagascar. Ankara de Diego-Suarez, *Service des Eaux et Forêts 9383* (holotype, P!).

Blotia ankaranae Leandri var. *sambiranensis* Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 243. 1957. TYPE: Madagascar. Bassin supérieur du Sambirano, 1700 m, *H. Humbert 18642* (lectotype, designated by Hoffmann & McPherson, 1998: 250, P!; duplicates, K!, P!).

Savia revoluta Scott-Elliot, J. Linn. Soc., Bot. 29: 48. 1891. Syn. nov. TYPE: Madagascar. Fort Dauphin, May, *G. E. Scott-Elliot 2558* (lectotype, designated here, K!; duplicates, BM!, E not seen, K!).

Savia bojeriana Baill. var. *perrieri* Leandri, Notul. Syst. (Paris) 7: 189. 1939. Syn. nov. TYPE: Madagascar. Forêt d'Analamazaotra, 800 m, *H. Perrier de la Bâthie 4647* (lectotype, designated here, P!; duplicate, P!).

IUCN Red List category. *Wielandia bemarensis* is widely distributed throughout the northwestern parts of Madagascar (Hoffmann & McPherson, 1998: fig. 4A). As with all species in Madagascar that occur predominantly in primary vegetation, there is cause for concern for its long-term survival, and it is rated NT (Near Threatened) by IUCN Red List criteria (2001).

For description, a complete list of specimens studied, and a distribution map see Hoffmann and McPherson (1998).

Selected specimens examined. MADAGASCAR. **Antsiranana prov.:** Diego-Suarez, Ankara, *Service Forestier 9383* (MO, P). **Mahajanga prov.:** Boina region, ca. 15 km NW of the village of Villanandro, RNI Namoroka, *DuPuy, DuPuy & Raharilala MB372* (K).

2. **Wielandia bojeriana** (Baill.) Petra Hoffm. & McPherson, comb. nov. Basionym: *Savia bojeriana* Baill., Adansonia 8: 345. 1868. TYPE: Madagascar. s. loc., *W. Bojer s.n.* (holotype, P!). Figures 1I–K; 3D–F; 4C; 6B; 7A; 8C; 9A.

Shrub, 2–7.5 m high; branches terete, striate, with lenticels, puberulent when young, glabrescent when older, rarely glabrous from the beginning. *Stipules* deltoid, acute, 1–2 × 0.5–1 mm, puberulent abaxially on apex and margins, caducous; *petioles* 1–3(–6) × 0.8–1.5(–2) mm, adaxially channeled to terete, puberulent or glabrous; petiolar vascular bundles cylindrical in cross section; *leaf blades* ovate, more rarely oblong, elliptic or nearly orbicular, apically acute to rounded, never with a dark point or mucro, basally acute to rounded, not to slightly revolute, with exmedial ultimate marginal venation, (1–)2–6(–16) × (0.5–)1–3(–6.5) cm, glabrous, rarely adaxially puberulent along the midvein (especially basally), coriaceous to chartaceous, green to yellowish green on both sides, dull to shiny, abaxially frequently slightly lighter, venation adaxially slightly (rarely more strongly) prominent, abaxially prominent; anticlinal walls of leaf epidermis straight; stomata with 3 to 4(to 6) subsidiary cells. *Inflorescences* axillary and cauline on the same individual. *Staminate flowers* (2–)5–10 × (2–)4–7 mm, often with green sepals, white (in young flowers) to maroon (in old flowers) petals and filaments, and yellow anthers fresh or dry, delicate, solitary or in 2- to 6-flowered fascicles; bracts deltoid, acute, keeled, up to 1 × 0.5 mm, abaxially puberulent; buds pedicellate, globose; *pedicels* 5–15 × 0.2–0.3 mm, terete, puberulent or glabrous; *sepals* 5, reflexed, oblong, rounded, 2–3 × 1.5–2 mm, ± fimbriate, glabrous or abaxially puberulent; *petals* 5, reflexed, rhombic, lanceolate or linear, acute or long-acuminate, (2–)3–5 × 1–3 mm, distinctly longer than the sepals, erose, glabrous, maroon; *disc* annular,

irregularly crenulate to lacerate, sometimes lacerate to the base, to 0.5 mm long, glabrous; *stamens* 5, (1–)3–4 mm long; filaments free, flattened to terete, (1–)2.5–3.5 × ca. 0.5 mm; anthers broadly ovoid, 0.2–0.5 mm long, glabrous, connective deltoid, basally up to 0.5 mm wide, thecae at an angle of 45°–90°; pistillode terete, ca. 1 × 0.3 mm, 3-fid for half or all of its length; the parts terete, distally pubescent or glabrous. *Pistillate flowers* with green sepals and ovaries, white (in young flowers) to maroon (in old flowers) petals (fresh or dry), axillary, solitary, rarely in twos, 4–5 × 4–8 mm, robust; bracts deltoid, acute, keeled, 1–2 × ca. 1 mm, abaxially puberulent; buds pedicellate, ellipsoid; *pedicels* 8–25 × 0.5–0.7 mm, terete to flattened, distally slightly thickened, puberulent or glabrous; *sepals* 5, usually reflexed, lanceolate to linear, acute, 2–5 × 2–3 mm, with ± membranous, fimbriate margins, otherwise glabrous, thick, usually with conspicuously prominent veins, light brown when dry; *petals* as in staminate flowers, but only reflexed as far as the thick sepals allow, caducous; *disc* as in staminate flowers, but ca. 1 mm long; *ovary* 3-locular, cylindrical to globose, ca. 1 mm long, glabrous; *styles* 3, 2-fid to the base; stylar branches terete, 2–3 × 0.2–0.3 mm, straight or basally ascending, then appearing eccentric with sometimes a conical structure between the styles, glabrous; stigmas obtuse to truncate. *Fruits* solitary, subglobose to globose, 3-lobed, 8–10 × 12–13 mm, glabrous, smooth, not reticulate, distinctly wrinkled when dry, yellow to light yellowish brown; fruiting pedicels 10–20 × 0.5–0.7 mm, terete, puberulent or glabrous; columella ca. 7 × 1 mm, basally abruptly thickened to 1.5 mm, distally hardly thickened or to 2 mm wide, disc and sepals persistent; exocarp ca. 0.5 mm thick, 3-layered; the middle layer fibrous (probably fleshy when fresh); endocarp ca. 0.3 mm thick; *seeds* 1 or 2 per locule, apiculate, with ± distinct perichalazal annulus, globose to ovoid (1-seeded locules) or nearly triangular in cross section (2-seeded locules), 5–9 × 5–6 mm, smooth, dull, ± marbled, greyish brown; embryo conical with hemi-ovoid cotyledons; radicle terminal, hardly longer than wide.

Illustrations: Leandri in Humbert, Fl. Madagascar 111(1): 123, figs. 20.6–8. 1958 (habit, fruits).

A Kew sheet (*Bojer s.n.* and s. loc.) of *Wielandia bojeriana* is a possible isotype.

In the protologue of *Savia revoluta* (Scott-Elliot, 1891) the syntypes *Scott-Elliott 2538* and *2845* are cited. These numbers are not present in BM, E, and K, and only number *2845* exists in P. The numbers *2558* (BM, E, K) and *2848* (K), however, are marked "Typus!" by N. E. Brown, who is acknowledged by Scott-Elliott (1891) for his help. This suggests that the latter are the correct types, and that the confusion is due to a typographic error. We choose number *2558* as lectotype, because duplicates exist in three different herbaria.

Both the type of *Savia bojeriana* var. *perrieri* and the specimen *Boiteau 132* have the bullate leaves said to distinguish var. *perrieri* from the typical variety (Leandri, 1939), but they are otherwise morphologically unremarkable in *Wielandia bojeriana*. Bullate leaves are also found on rare occasions in *W. oblongifolia*.

Wielandia bojeriana is a morphologically variable species, especially in leaf shape, size, and texture. The two specimens *Schatz et al. 1844* (P) and *Schatz & Gentry 2080* (P), although perfectly matching other specimens of *W. bojeriana* in their flower characters, are vegetatively different in having acuminate leaves and being completely glabrous. In view of the considerable variability of this species, we have refrained from describing a new taxon exclusively based on foliar characters in two specimens.

Distribution and ecology. Madagascar, central to western limit of eastern floral domain, rarely in southwestern domain. Shady coastal forest, dry forest and scrub; on sand over granite and basalt; 0–1600 m altitude. Can be very common in wet forest (observed in Ivohibe). Figure 10.

IUCN Red List category. *Wielandia bojeriana* is relatively frequent in healthy forests and is protected in several nature reserves. However, as the remaining primary vegetation is disappearing fast in Madagascar, we recommend that it be rated, by IUCN (2001) criteria, NT (Near Threatened); 47 specimens were studied.

Etymology. The species epithet refers to the collector of the type specimen, W. Bojer (1797–1856).

Vernacular names. Hazomainty (*Boiteau 2507*), Zaimboalavo (or Zaunboalavo?) (*Rakotoniaina 10722-RN, 11712-RN*).

Representative specimens examined. MADAGASCAR. **Antsiranana prov.:** vallée de la Beandrona, à l'Est d'Ambanja, *Capuron 18930-SF* (K, P). **Toamasina prov.:** Forêt de Sahavolamena, au Sud de Soanierana-Ivongo, *Capuron 28116-SF* (P); Nosy Mangabe, *Schatz et al. 1844* (MO, P); Forêt près de Périnet, *Boiteau 132* (K, MO, P); Tsinjoarivo, 45 km à l'Est d'Ambatolampy, *Capuron 18510-SF* (K, MO, P); Ilot boisé du sommet de la montagne appelée Analavorikely, *Cours 2138* (MO, P); Distr. Ambatondrazaka, Lac Alaotra, Menaloha, *Cours 636* (= TAN 4119 = MEN 20) (P). **Fianarantsoa prov.:** Ambalavao, Sendrisoa, *Bosser 1195* (P); Réserve Spéciale de Pic d'Ivohibe, Forest of Marovitsika, *Hoffmann et al. 238* (DAV, G, K, MO, P, TAN). **Toliara prov.:** Forêt d'Analavelona (Bassin du Fiherenana), *Humbert 19759* (K, MO, P); vallée moyenne du Mandrare près d'Anadabolava, *Humbert 12501* (P); préfecture de Tolanaro (Ft. Dauphin), Petriky, *Rabevohitra et al. 1816*

(K, MO, P); Toliara, prefecture de Tolanaro (Fort Dauphin), Mandena, *Rabevohitra 2130* (K, MO, P); Forêt du Vinanibe, près de Ft. Dauphin, *Capuron 22291-SF* (MO, P); Versant sud et plateau sommital du massif granitique du Vohitrandriana (au S de Ranopiso), *Capuron 28582-SF* (MO, P).

3. Wielandia danguyana (Leandri) Petra Hoffm. & McPherson, comb. nov. Basionym: *Savia danguyana* Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 239. 1957. TYPE: Madagascar. Antsakatsaka, entre Beravina et Périnet, 14 Dec. 1951, *Service des Eaux et Forêts 5728* (lectotype, designated here, P!; duplicates, K!, MO!). Figures 1D–F; 5A, B; 6E, I; 7D; 8G; 9D; 13.

Blotia oblongifolia (Baill.) Leandri var. *louvelii* Leandri, Notul. Syst. (Paris) 7: 190. 1939 [as "louveli"]. Syn. nov. TYPE: Madagascar. Analamazaotra, 1925, *M. Louvel 77* (holotype, P!).

Small tree, 3–8 m high; indumentum absent; branches terete, striate. *Stipules* very early caducous; stipule scars visible even on youngest leaves; *petioles* 4–10 × 0.8–1.2 mm, adaxially slightly channeled; petiolar vascular bundles in cross section U-shaped with inflexed sides; *leaf blades* ovate, apically acuminate, apiculum rounded, basally acute to obtuse, 3–8 × 1.5–4.5 cm, coriaceous, yellowish green, slightly shiny, midvein adaxially strongly impressed, abaxially prominent, remaining venation prominent on both sides, conspicuously reticulate, distinct secondary veins absent; anticlinal walls of leaf epidermis straight; stomata with 3 to 4(to 6) subsidiary cells. *Inflorescences* axillary; either 3–5 cm long, unbranched, leafless, with fascicles of 2 to 5 staminate and pistillate flowers, a total of 25 to 40 flowers per inflorescence; or flowers in fascicles of 5 to 25 flowers in the axil of leaf-like bracts, appearing cauline on older branches after abscission of bracts. *Staminate flowers* up to 2 × ca. 2 mm, delicate; bracts deltoid, acute, ca. 0.5 × 0.5 mm; buds pedicellate, globose, completely encased by the sepals, petals strongly folded; *pedicels* 6–17 × 0.3–0.5 mm, terete, distally not thickened; *sepals* 5, deltoid, acute, ca. 1 × 0.5 mm; *petals* 5, spathulate, rounded, apically usually bent inward, clawed, ca. 1.5 × 0.5 mm; *disc* deeply alternipetalously lobed; lobes 5, 2-fid, erose or truncate, ca. 1 × 0.5 mm, hard; *stamens* 5; ca. 0.8 mm long; filaments free, terete, ca. 0.5 mm long; anthers ovoid, 0.3–0.5 mm long; pistillode terete, clavate, 3-lobed, ca. 0.8 × 0.5 mm. *Pistillate flowers* 2–3 × 1–2 mm, robust; bracts as in staminate flowers; buds pedicellate, ellipsoid, not encased by the sepals but by the thick petals; *pedicels* 4–10 × 0.5–0.7 mm, terete, distally thickened; *sepals* 5, deltoid, acute, ca. 1 × 1 mm, basally thick, margin membranous; *petals* 5, narrowly spathulate to rhombic, rounded to acute, 1.5–2 × ca. 0.5 mm, twice as long as sepals, thick; *disc* deeply alternipetalously lobed; lobes 5, emarginate, truncate or rounded, 0.5–0.8 × 0.2–0.5 mm; *ovary* 3-locular, globose, ca. 1 mm long; styles 3, 2-fid to the base; stylar branches terete, ca. 0.2 mm long, less than 0.1 mm thick, recurved; stigmas truncate; fruiting pedicels 8–15 × 0.7–1 mm, terete; calyx and disc usually persistent; columella 6–7 × 1 mm, basally hardly thickened, distally thickened to 1.5 mm. Only fragments of one *fruit* known, estimated diameter 8–9 mm, endocarp ca. 0.3 mm thick; *seeds* probably one per locule (only a single seed known), almost lens-shaped, 8 × 8 × 4 mm, smooth, shiny, dark brown; embryo lens-shaped with hemispherical cotyledons; radicle terminal, hardly longer than wide.

Illustrations: Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 241, figs. 7.1–7. 1957 (habit, flower), in Humbert, Fl. Madagascar 111(1): 127, figs. 21.1–7. 1958 (habit, flower; a more elaborate version of the same drawings); Levin, Ann. Missouri Bot. Gard. 73: 44, figs. 16, 17. 1986 (leaf venation); Muller et al., Trop. Subtrop. Pflanzenwelt 67: 63, figs. 1–6. 1989 (pollen).

The Paris sheet of *Service des Eaux et Forêts 5728* is designated as lectotype because Leandri was staff at the Paris herbarium.

Leandri (1958) must have described *Blotia oblongifolia* var. *louvelii* by mistake. He cited all three specimens listed for this variety (*Louvel 77*, *Gouvernement de Madagascar 9*, *Service de Colonisation 59*) on page 120 of the same volume under the morphologically distinctive species *Savia danguyana*, which they match perfectly.

Distribution and ecology. Eastern Madagascar, along Route Nationale No. 2 between Moramanga and Andasibe (Périnet). Forest. Figure 11.

IUCN Red List category. *Wielandia danguyana* is probably the most highly endangered species in the genus. No more than 11 specimens seem to have been collected, and the most recent collection was made in 1969. To judge from the herbarium specimens, the plants seem to flower profusely but to set fruit rarely. The species is restricted to a small area along a 16-km stretch of the road linking the capital Antananarivo with Toamasina (Tamatave), the largest port city of Madagascar. The land in this area is today intensely cultivated and virtually devoid of primary vegetation. The survival of this distinctive species depends entirely on the small nature reserve of Andasibe (Périnet) (ca. 8 km^2), and possibly the Parc National de Mantadia (ca. 102 km^2) close by. Both areas

Figure 13. *Wielandia danguyana*. —A. Detail of leaf venation, abaxial surface. —B. Flowering branches. —C. Seed, ventral view. —D. Embryo, lateral view. —E. Embryo, ventral view. —F. Columella, persistent after fruit dehiscence. —G. Pistillate flower. —H. Pistillate flower, sepals and petals partly removed. —I. Staminate flower. A, B, G–I, *Ramaroson 5728-SF* (K); C–F, *Jarson Paul 14988-SF* (K). Scale bars = 5 mm in A; 20 mm in B; 2 mm in C–I. Drawn by Catherine Wardrop.

are accessible for recreation and ecotourism, and under particular pressure because of their proximity to the capital Antananarivo. The plant was not found during a recent visit of the park by the first author.

Due to the lack of recent collections, locality data are vague and many only indicate the greater area of Moramanga, which is the district capital. The plant is known from only three precise localities: Analamazaotra, Périnet, and Antsakatsaka, the first two being but 5 km apart, and used as two different names for the same forest. The extent of occurrence that can be calculated from these data is a maximum of 250 km^2 (generously assuming that it was actually collected near the city of Moramanga, and assuming a 10-km width of the possible distribution area along the road). For these reasons, the species is here rated Endangered: EN B1ab(iii). The IUCN (2001) rating is very close to Critically Endangered because the area of occupancy for the three safely located collections could be calculated as only 7.68 km^2. However, there is a possibility that the collections with vague localities were made in different locations within the extent of occurrence indicated above.

Etymology. The epithet honors the French botanist P. A. Danguy (1862–1942).

Vernacular names. Fanjavala (Leandri 1958: 121; *d'Alleizette s.n., Gouvernement de Madagascar 9, Louvel 77, Service de Colonisation 59, SF 2529, SF 5728, SF 14988, SF 26834*).

Representative specimens examined. MADAGASCAR. **Toamasina prov.:** Périnet-Analamazaotra, *Capuron 11964-SF* (K, MO, P); PK 27–28 route Moramanga–Anosibenala, *SF 26834* (MO, P).

4. **Wielandia elegans** Baill., Étude Euphorb., Atlas: 40, t. 22. 1858. *Savia elegans* (Baill.) Müll. Arg., Linnaea 32: 78. 1863. TYPE: [Seychelles.] Île Carenga, "communiqué par Boivin," 12 Nov. 1841, *A. Perrillé 971* (holotype, P!).

Wielandia elegans Baill. var. *perrieri* Leandri, Notul. Syst. (Paris) 7: 190. 1939. TYPE: Madagascar. Domaine de l'Ouest, Anbongo, Manongarivo, bois sablonneux, Jan. 1904, *H. Perrier de la Bâthie 1683* (lectotype, designated by Hoffmann, 1998: 335, P!; duplicate, P!).

IUCN Red List category. *Wielandia elegans* is distributed more or less evenly throughout Madagascar and also occurs in Comoro Islands (Mayotte) and the Seychelles (Hoffmann, 1998: fig. 2). Despite this wide distribution, it is not common and is reported to be in decline in some areas (Matyot, 2005). Considering the general threat to primary vegetation in the region, we propose the IUCN (2001) rating NT (Near Threatened) for this species.

For description, list of specimens studied, and distribution map see Hoffmann (1998).

5. **Wielandia fadenii** (Radcl.-Sm.) Petra Hoffm. & McPherson, comb. nov. Basionym: *Savia fadenii* Radcl.-Sm., Kew Bull. 27: 508. 1972. *Petalodiscus fadenii* (Radcl.-Sm.) Radcl.-Sm., Kew Bull. 47: 679. 1992. TYPE: Kenya. Kilifi District, Chasimba, ca. 13 km NE of Kaloleni, Kaloleni–Kilifi rd., 03°43′S, 39°42′E, 200 m, *R. B. Faden, A. Evans & M. Githui 71/263* (holotype, K!; isotype, EA not seen). Figures 1G, H; 2D–F; 3G, H; 5C, D; 6A; 7E; 8B; 9E.

Tree or shrub, 1.5–15 m high, trunk sometimes fluted or gnarled; indumentum absent except for floral disc; bark smooth; branches terete, ± striate. *Stipules* very early caducous (bracts can easily be mistaken for stipules), ovate, obtuse to rounded, auriculate, ca. 1.5 × 1 mm; *petioles* 3–5 × 0.5–1 mm, adaxially channeled; petiolar vascular bundles in cross section shallowly U-shaped; *leaf blades* ovate, apically long-acuminate, apiculum rounded, basally obtuse to truncate, rarely acute, often slightly decurrent, (2.5–)4–8 × (1–)2–4 cm, chartaceous to coriaceous, yellowish green to olive-green, adaxially moderately shiny, abaxially dull to slightly shiny, midvein adaxially hardly prominent, abaxially prominent, remaining venation prominent on both sides; anticlinal walls of leaf epidermis undulating; stomata with 3(or 4) subsidiary cells. *Inflorescences* axillary, 1–2 cm long, unbranched, leafless (rarely some strongly reduced bracts present); 1(2) pistillate and 2 to 4 staminate flowers per fascicle; 15 to 25 flowers per inflorescence. *Staminate flowers* ca. 2 × 2–4 mm, delicate; bracts deltoid, acute, erose to fimbriate, ca. 0.5 × 0.5 mm; buds pedicellate, globose; *pedicels* 7–15 × 0.1–0.2 mm, terete, distally not thickened; *sepals* 5, narrowly deltoid to lanceolate, acute to obtuse, undulate, slightly erose, ca. 1 × 0.5 mm; *petals* 5, lanceolate, acute, undulate, strongly erose, clawed, 1.5–2 × 0.5–1 mm, about twice as long as sepals; *disc* deeply lobed; disc lobes 5, alternipetalous, emarginate, truncate or rounded, 0.5–1 × 0.3–0.5 mm, as long as or slightly shorter than the sepals, shortly and densely pubescent; *stamens* 5, ca. 1 mm long; filaments free, terete, ca. 0.8 mm long; anthers ellipsoid, ca. 0.2 mm long; pistillode 3-fid, ca. 0.2 × 0.2 mm. *Pistillate flowers* 2–3 × 2–3 mm, delicate; bracts and buds as in staminate flowers; *pedicels* (0.5–)2–7 × 0.3–0.5 mm, terete, distally slightly thickened; *sepals* 5, deltoid, acute to obtuse, undulate, 1–1.5 × ca. 1 mm, erose; *petals* as in staminate flowers, but less strongly erose; *disc* as in staminate flowers, but lobes distinctly 2-fid; *ovary* globose to

ovoid, 3(or 4)-locular, 3(or 4)-lobed, ca. 1 mm long; styles 3(or 4), 2-fid to the base; stylar branches terete, ca. 0.5 × 0.1–0.2 mm, ± straight, spreading to horizontal; stigmas acute. *Fruits* solitary, subglobose, 3-lobed, ca. 9 × 12–14 mm, strongly to hardly reticulate, light yellowish brown; fruiting pedicels 2–7 × 0.5–0.6 mm, terete; columella 5–6 × ca. 1 mm, basally hardly thickened, distally thickened to 1.5 mm; exocarp ca. 0.3 mm thick; endocarp 0.3–0.5 mm thick; *seeds* 1 per locule, 7–8 × 5–6 mm, smooth, shiny, ± marbled, reddish brown; embryo with thin, folded cotyledons; radicle about 3× as long as wide.

Illustrations: Radcl.-Sm. in Polhill, Fl. Trop. East Afr., Euphorb. 1: 84, figs. 11.1–5. 1987 (habit, flowers, fruits); Beentje, Kenya Trees, Shrubs & Lianas: 222. 1994 (habit).

SF 12398 (P) is the only specimen of *Wielandia fadenii* from southeast Madagascar. It is vegetatively identical to other collections of *W. fadenii*, although the leaves are thicker and dry a more yellowish green. It differs, however, in having smaller flowers with smaller, thicker, entire, spathulate, rounded to truncate petals (1 × 0.5 mm) as long as (staminate) or half as long as (pistillate) the sepals, shorter disc lobes and stamens (0.5 mm), and a larger, clavate pistillode (0.5 × 0.5 mm). It might represent a new subspecies.

Distribution and ecology. Southeast Kenya, Mayotte (Comoro Islands), and northwest Madagascar; one morphologically and geographically distinct collection from the southeast coast of Madagascar. Evergreen and seasonal forests, riverine vegetation, dry bush; on limestone in Kenya and Madagascar (whereas Mayotte is of volcanic origin); at 0–600 m altitude. Figure 12.

IUCN Red List category. The distribution area of *Wielandia fadenii* is extensive and includes three different countries. A total of 32 specimens (19 from Madagascar, two from Mayotte, and 11 from Kenya) was studied, only a few of which had developed seeds. We recommend that it be assigned, by IUCN (2001) criteria, the category Near Threatened (NT) because of deforestation throughout its range.

Etymology. The species epithet honors the senior collector of the type, Robert B. Faden.

Vernacular names. Madagascar. Fanjavala (*Labat & Deroin 2275*), Menahy (*SF 12398*), Meridina (*Humbert 18932*), Salaza (*Randrianamera? 89-RN*), Tepapanga (*Humbert 18932*), Vagmo (*SF 8256*), Velovoaraiki (*Randrianamera? 9725-RN*), Velovoaraiky (*Randrianamera? 9718-RN*).

Representative specimens examined. KENYA. Kilifi District, Chonyi Rock, 9 km NE of Kaloleni, *Bally & Smith B 14396* (K, MO); side branch of Ndzovuni river just N of ford 7 km from Mtsengo, *Luke & Robertson 6086* (K, MO); Kwale District, Chale Peninsula, *Luke 2888* (K). COMORO ISLANDS. Mayotte, Hachiroungou, *Pascal 503* (K, MO, P); Hachiroungou, *Pascal 763* (K, P). MADAGASCAR. **Antsiranana prov.:** Forêt de Sahafary, bassin de la Saharaina, *Capuron 20978-SF* (K, MO, P); Massif de l'Anjanabe, au N d'Antongondriha (bassin de l'Androranga, affluent de Bemarivo), *Capuron 773-SF* (MO, P); Massif de l'Ankarana, près d'Ambondromifehy, *Capuron 22027-SF* (K, MO, P). **Mahajanga prov.:** SW d'Antsalova, Forêt et Savoka de Tsimembo vers Tsaranandakana, Ankilimioky et Ambereny, *Capuron et al. 2312* (K, MO, P); au dessus de l'Ambodiria, 1 km a l'E d'Ambinda (RN9, près d'Antsalova), *Labat & Deroin 2275* (K, MO, P); Forêt de Tsiampihy et forêts côtières près de Besaraha de Bemiha et de Soahanina, *Leandri 2286* (P); Soalala, Andranomavo, RN 8, *Rakotovao 5690-RN* (P).

6. Wielandia laureola (Baill.) Petra Hoffm. & McPherson, comb. nov. Basionym: *Savia laureola* Baill., Adansonia 2: 33. 1861. *Petalodiscus laureola* (Baill.) Pax in Engl. & Prantl, Nat. Pflanzenfam. 3(5): 15. 1890. TYPE: Madagascar. s. loc., *L. M. A. Du Petit-Thouars s.n.* (lectotype, designated here, P!; duplicate, P!). Figures 1A–C; 4D, E; 6G, H; 7C; 8F; 9C.

Tree or shrub, 2–10 m high; indumentum absent; branches flattened or winged to angular when young, later terete. *Stipules* deltoid to ovate, acute, 0.5–1.5 × 0.5–0.7 mm, often persistent; *petioles* 2–5 × 0.8–1.5 m, adaxially channeled; petiolar vascular bundles basally U- to V-shaped in cross section, cylindrical from the middle to the distal end; *leaf blades* ovate to elliptic, apically acute to slightly acuminate, very rarely with subapical glandular thickening, basally acute to rounded, frequently (especially basally) slightly revolute, 2.5–6(–8.5) × 1–4 cm, coriaceous, adaxially green to greyish green, shiny, abaxially darker, reddish grey-green, dull to slightly shiny, midvein adaxially prominent, forming a thin undulating crest, abaxially slightly prominent, remaining venation adaxially not to hardly prominent, abaxially prominent, leaf size decreasing gradually toward the tips of the branches; anticlinal walls of leaf epidermis straight; stomata with 3(to 5) subsidiary cells. *Inflorescences* axillary; basal flowers in the axil of normal or moderately reduced foliage leaves; distal flowers in (sometimes branched) inflorescences up to 6 cm long; branches frequently subtended by reduced, subulate leaves, distally with numerous scale-like bracts; staminate and pistillate flowers in the same inflorescence. *Staminate flowers* in fascicles of up to 10(rarely up to 40) flowers; flowers up to 1 × 2.5 mm, delicate; bracts deltoid to ovate, acute, ca. 0.5 × 0.5 mm, shiny reddish brown; buds shortly pedicellate, globose to ellipsoid; *pedicels* ca. 2 × 0.2 mm, terete; *sepals* 5, 1–1.5 × 0.3–0.5 mm; the outer two oblong, rounded; the inner petaloid,

spathulate, with membranous margin, apically erose; *petals* 5, spathulate to rhombic, acute to rounded, clawed, erose, ca. 1 × 0.2–0.3 mm; *disc* deeply alternipetalously lobed; lobes 5, 2-fid, ca. 0.5 × 0.2 mm; *stamens* 5, ca. 0.8 mm long; filaments basally fused to ca. 2/3 of their length, terete, ca. 0.6 mm long; anthers ovoid, ca. 0.2 mm long; pistillode 3-fid, ca. 0.2 × 0.2 mm. *Pistillate flowers* solitary, up to 2 × 2 mm, robust; bracts as in staminate flowers; buds as in staminate flowers, but shorter and with thicker pedicels; *pedicels* 1–3 × 0.5–0.8 mm, terete; *sepals* 5, 1.5–2 × ca. 1 mm thick; the outer two deltoid, acute to obtuse; the inner three oblong, rounded, with membranous margin; *petals* 5, spathulate to rhombic, acute to rounded, erose, 1.2–1.5 × ca. 0.5 mm, hardly shorter than the sepals; *disc* consisting of discrete lobes; lobes 5, petaloid, alternipetalous, truncate, erose, ca. 0.8 × 0.5 mm, delicate; *ovary* 3-locular, ovoid, ca. 1 mm long; styles 3, 2-fid to the base; stylar branches terete, ca. 0.3 × 0.1 mm, slightly spreading, recurved; stigmas truncate. *Fruits* solitary, smooth, not reticulate but somewhat wrinkled when dry, yellow to medium brown; only dehisced fruits known; fruiting pedicels 2–3 mm long; columella 6 × 0.5 mm, distally only slightly thickened; disc and perianth persistent; exocarp 0.1–1 mm thick, brittle to coriaceous, 3-layered; the middle layer fibrous (fleshy when fresh?); endocarp ca. 0.3 mm thick; *mature seeds* unknown, apparently one per locule, immature seed 5–6 × 3–6 mm.

Illustrations: Leandri in Humbert, Fl. Madagascar 111(1): 117, figs. 19.7–8. 1958 (habit).

One of the two eligible sheets (Baillon, 1861) of *Du Petit-Thouars s.n.* in P was chosen as lectotype of *Wielandia laureola* because it bears floral diagrams drawn by Baillon.

Distribution and ecology. Madagascar, restricted to area around Tamatave (east coast). The specimen *Forbes s.n.*, Mozambique, without further locality, at Kew is certainly also from Madagascar but has been erroneously labeled. Littoral forest on sand from sea level to 50 m altitude. Figure 10.

IUCN Red List category. *Wielandia laureola* is an endangered species occurring exclusively in coastal white sand forest. It is particularly vulnerable to subsistence logging by the local population because it is slow-growing. All white sand forests of Madagascar are under extreme threat. The second author is presently leading a study to determine the extent and species composition of the remaining eastern forests of this type. Conservation decisions based on the results will improve the chances of survival of species such as this one. Sixteen specimens of *W. laureola* were located in the course of this study, only one of which bears fruit (but no mature seed). The distribution area is a ca. 400-km-long stretch of coast with a maximum width of white sand forest of 5 km. Actual extent of occurrence is therefore no more than 2000 km^2. The species has been found in only seven locations (Foulpointe, Analalava, Tampina, Ambila-Lemaitso, Andovoranto, Pointe à Larree, Ataimby), the first two being but 2 km apart. None of its range is included in protected areas, and it is severely fragmented in the middle by the city of Toamasina (Tamatave), the major port of Madagascar, which is highly polluted. We therefore suggest that this species be assigned the IUCN (2001) rating Endangered: EN B1ab(iii).

Etymology. The species was probably named for the resemblance of its leaves to bay leaf, *Laurus nobilis* L., Lauraceae (Latin, laureola = a small branch of laurel, especially as to announce a victory). The epithet is a noun in apposition, not an adjective.

Vernacular names. Berando (*SF 1104*), Fanamorano (Leandri, 1958: 119; *SF 12918*), Fanazava (*SF 2943*), Maroando (*SF 2983*).

Uses. Against earache (*Martine C 16*).

Representative specimens examined. MADAGASCAR. **Toamasina prov.:** Forêt de Mangalimaso, à l'Ouest de Foulpointe, *Capuron 22083-SF* (K, MO, P); Ambila, S de Tamatave, *Decary 6536* (A, G, P, S); Tampina, près Tamatave, *Lam & Meeuse 6042* (K, MO, P); **Fianarantsoa prov.:** Antaimby, *Ranaivojaona 655* (MO, P).

7. **Wielandia leandriana** (Petra Hoffm. & McPherson) Petra Hoffm. & McPherson, comb. nov. Basionym: *Blotia leandriana* Petra Hoffm. & McPherson, Novon 7: 249. 1997. TYPE: Madagascar. Toliara prov.: Tolagnaro (Ft. Dauphin), NE of Mandena, coastal forest, roadside E & beyond QIT camp, 25 m, 7 Dec. 1989, *G. McPherson & N. Dumetz 14651* (holotype, MO!; isotypes, B!, DAV!, K!, P!, TAN!, TEF!).

IUCN Red List category. *Wielandia leandriana* is restricted to the east coast of Madagascar but has a wide latitudinal range (Hoffmann & McPherson, 1998: fig. 4A). It is known from only a limited number of collections. Considering the general threat to the eastern forests from tavy and subsistence logging, we suggest for it the IUCN (2001) rating NT (Near Threatened).

For description, list of specimens studied, and distribution map see Hoffmann and McPherson (1998).

8. **Wielandia mimosoides** (Baill.) Petra Hoffm. & McPherson, comb. nov. Basionym: *Savia mimo-*

soides Baill., Adansonia 2: 34. 1861. *Petalodiscus mimosoides* (Baill.) Pax in Engl. & Prantl, Nat. Pflanzenfam. 3(5): 15. 1890. *Blotia mimosoides* (Baill.) Petra Hoffm. & McPherson, Adansonia, sér. 3, 20(2): 253. 1998. TYPE: Madagascar. s. loc., *L. M. A. Du Petit-Thouars s.n.* (lectotype, designated by Hoffmann & McPherson, 1998: 253, P!).

Savia hildebrandtii Baill. in Grandid., Hist. Phys. Madagascar, Plantes 4 (Atlas 2): t. 209. 1892. *Blotia hildebrandtii* (Baill.) Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 242. 1957. TYPE: Baillon in Grandidier, 1892: t. 209. 1892.
[The specimen *Hildebrandt 3931* (P), collected in 1881, bears a strong resemblance to the type and was likely the basis for the plate.]

Savia maroando Danguy ex Lecomte, Bois d'Analamazaotra: 71. 1922. TYPE: Madagascar. Analamazaotra, *E. Thouvenot* (*coll. Ramanantsvolana*) *131* (lectotype, designated by Hoffmann & McPherson, 1998: 254, P!; duplicates, BM!, K!, P!, US!).

IUCN Red List category. *Wielandia mimosoides* is fairly widespread in the eastern half of Madagascar (Hoffmann & McPherson, 1998: fig. 4B) but not indicated from secondary habitats. The general threat to and negative projection for all primary vegetation of Madagascar necessitate that this species receives the IUCN (2001) rating of NT (Near Threatened).

For description, a complete list of specimens studied, and a distribution map see Hoffmann and McPherson (1998).

Selected specimens examined. MADAGASCAR. **Toamasina prov.:** Mangabe, Ampasimaventy, Andranomanapika, *Service Forestier 26166* (MO, P); Analamazaotra, *Thouvenot 131* (BM, K, P, US). **Toliara prov.:** Didy à Brickaville, *Cours 4716* (K, P).

9. Wielandia oblongifolia (Baill.) Petra Hoffm. & McPherson, comb. nov. Basionym: *Savia oblongifolia* Baill., Adansonia 2: 35. 1861. *Petalodiscus oblongifolius* (Baill.) Pax in Engl. & Prantl., Nat. Pflanzenfam. 3(5): 15. 1890 [as "oblongifolia"]. *Blotia oblongifolia* (Baill.) Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 240. 1957. TYPE: Madagascar. Ste.-Marie de Madagascar, fôret de Tafondrou, *L.-H. Boivin 1886*[5] (lectotype, designated by Hoffmann & McPherson, 1998: 257, P!; duplicate, P!).

Savia decaryi Leandri, Bull. Soc. Bot. France 81: 589. 1934. *Savia oblongifolia* Baill. var. *decaryi* Leandri, Notul. Syst. (Paris) 7: 190. 1939. TYPE: Madagascar. Ambila, au sud du Tamatave, *R. Decary 6389* (lectotype, designated by Hoffmann & McPherson, 1998: 257, P!; duplicates, K!, US!).

IUCN Red List category. *Wielandia oblongifolia* has been collected in a large area comprising the humid eastern bioclimatic zone and the transition from humid to subhumid zones (Hoffmann & McPherson, 1998: fig. 4C). Threats to its survival come from continuing deforestation and degradation of primary habitats, and the species is here rated NT (Near Threatened) by IUCN (2001) criteria.

The first mention of this species in print was the nomen nudum *Wielandia oblongifolia* Baill. (1858: 569).

For description, a complete list of specimens studied, and a distribution map see Hoffmann and McPherson (1998).

Selected specimens examined. MADAGASCAR. **Toamasina prov.:** Ambila, S de Tamatave, *Decary 6389* (K, P, US); Analamazaotra, *Thouvenot 1918* (K, P).

10. Wielandia platyrachis (Baill.) Petra Hoffm. & McPherson, comb. nov. Basionym: *Petalodiscus platyrachis* Baill., Étude Euphorb., Atlas: 40, t. 22. 1858 [as "platyrachys"]. *Savia platyrachis* (Baill.) Baill., Adansonia 2: 33. 1861. TYPE: "Madagascar?". s. loc., *L. M. A. Du Petit-Thouars s.n.* (lectotype, designated here, P!; duplicate, P!). Figures 1L–N; 2A–C; 3I; 4F, G; 6C, D, K; 7B; 8D; 9B.

Savia pulchella Baill., Adansonia 2: 35. 1861. *Petalodiscus pulchellus* (Baill.) Pax in Engl. & Prantl, Nat. Pflanzenfam. 3(5): 15. 1890 [as "pulchella"]. Syn. nov. TYPE: "Madagascar? Arbuste du jardin de Saint-Denis de la Réunion, venu de graines rapportées de Sainte-Marie de Madagascar par Richard," *L.-H. Boivin 1886*[3] (lectotype, designated here, P!; duplicate, P!).

Savia andringitrana Leandri, Bull. Soc. Bot. France 81: 587. 1934. Syn. nov. TYPE: Madagascar. Base nord du Pic d'Ivohibe, 24 Sep. 1926, *R. Decary 5484* (lectotype, designated here, P!; duplicates, K!, P!, US!).

Savia andringitrana Leandri var. *micrantha* Leandri, Bull. Soc. Bot. France 81: 587. 1934. Syn. nov. TYPE: Madagascar. Prov. Farafangana: Karianga, en forêt, 5 Oct. 1926, *R. Decary 5556* (holotype, P!; duplicate, BM!, K!, S!, US!).

Savia platyrachis (Baill.) Baill. var. *microphylla* Leandri, in Humbert, Fl. Madagascar 111(1): 120. 1958. Syn. nov. TYPE: Madagascar. Forêt d'Analamihilana, 850 m, 27 Dec. 1944, *G. Cours 1998* (holotype, P!).

Tree or shrub, 2–10 m high, to 1 m diameter; indumentum absent; young branches flat or winged, later terete, with lenticels. *Stipules* deltoid to ovate, acute, ± auriculate, (0.5–)1–2(–3) × 0.5–1 mm, usually persistent; *petioles* 2–5(–10) × 0.5–2 mm, adaxially channeled; petiolar vascular bundles basally in cross section U-shaped with inflexed sides, cylindrical from the middle to the distal end; *leaf blades* ovate to elliptic or oblong, apically acuminate

to acute, usually distinctly dark-mucronate, basally decurrent, acute, obtuse, or rounded, often slightly auriculate, not to distinctly revolute, (1.5–)4–9(–13.5) × (0.5–)1–4(–6) cm, coriaceous to chartaceous, discolorous, adaxially greyish green, yellowish green, or olive-green, abaxially dark red to reddish brown, dull to slightly shiny on both sides; midvein adaxially prominent, forming a thin undulating crest, abaxially prominent; remaining venation slightly prominent on both sides; anticlinal walls of leaf epidermis straight; stomata with 3(or 4) subsidiary cells. *Inflorescences* terminal, axillary and cauline on the same plant, staminate and pistillate flowers in separate fascicles of 2 to 10 flowers, axillary inflorescences either 1–25 cm long, unbranched, leafless, or flowers in the axil of leaf-like bracts, transition between bracts and foliage leaves subtending flowers gradual, sometimes individual foliage leaves present in otherwise leafless inflorescences; bracts deltoid, acute, to 0.5 × 0.5 mm; buds pedicellate, globose to ellipsoid. *Staminate flowers* orange to red when fresh, maroon with yellow anthers when dry, 2–3 × 4–6 mm, robust; *pedicels* 5–12 × 0.2–0.3 mm, terete; *sepals* 5, deltoid to oblong, acute to rounded, 1–2 × ca. 1 mm; *petals* 5, orbicular to lanceolate, rounded to obtuse, clawed; the base with a distinct adaxial thickening; the margin sometimes narrowly membranous, 2–4 × 2–3 mm, about twice as long as the sepals; *disc* obscured by basal thickening of petals, ca. 0.2 mm long, annular, often with small acuminate lobes between the petals, entire to erose; *stamens* 5, 1–1.5 mm long, often no longer than the base of the petals; filaments free, leaning toward the center, terete, ca. 1 mm long (see note); anthers ovoid, close together (often giving the appearance of connate filaments), introrse, outward-pendulous, ca. 0.2 mm long; pistillode terete, 0.2–0.3 × ca. 0.2 mm, deeply 3-fid; the parts channeled inside. *Pistillate flowers* orange or red when fresh, maroon when dry, 4–5 × 5–7 mm, robust; *pedicels* 6–18 × 0.5–0.7 mm, terete, distally thickened; *sepals* 5, deltoid to oblong, acute to rounded, 1–2 × ca. 1 mm; *petals* 5, ± orbicular, rounded to obtuse, clawed; the base with a distinct adaxial thickening; the margin sometimes narrowly membranous, 3–4 × 3–4 mm, 2 to 3× as long as sepals; *disc* extending to the same length as the basal thickening of the petals, or obscured by them, ca. 0.5 mm long, annular, often with small acuminate lobes between the petals, lacerate, erose or entire; *ovary* 3-locular, ovoid, ca. 1 mm long; styles 3, 2-fid to the base or nearly so; stylar branches terete, ca. 0.5 × 0.2 mm, erect to recurved; stigmas truncate to slightly capitate. *Fruits* solitary (only one flower per fascicle reaching maturity), nearly globose, 3-lobed, ca. 8 × 10–14 mm, reticulate, smooth, medium to dark brown; fruiting pedicels (5–)7–12 × 0.4–1 mm, terete; columella ca. 7 × 1 mm, basally thickened to 1.5 mm, distally hardly thickened or up to 1.5 mm wide; disc and sepals persistent; exocarp ca. 0.2 mm thick; endocarp ca. 0.3 mm thick; *seeds* 1 or 2 per locule, apiculate, with ± distinct perichalazal annulus, globose to ovoid, 5–7 × 6–7 mm (1-seeded locules) or nearly triangular in cross section, 5–7 × 4–5 mm (2-seeded locules), rough, ± marbled, greyish brown to dark brown; embryo ovoid with hemiovoid cotyledons; radicle terminal, hardly longer than wide.

Illustrations: Baill., Étude Euphorb.: t. 22, figs. 11–14. 1858, reproduced in Pax in Engl. & Prantl, Nat. Pflanzenfam. 3(5): 16. 1890, Pax & K. Hoffm. in Engl., Pflanzenr. 81: 174. 1922, Nat. Pflanzenfam. ed. 2, 19c: 67. 1931 (flower). This drawing shows some characters that are atypical of *W. platyrachis*, e.g., an extremely long disc and filaments and the absence of the basal thickening of the petals, which is clearly visible in the type specimen. In addition, the drawn petals and sepals differ in outline from what we saw on the specimen. Leandri in Humbert, Fl. Madagascar 111(1): 123, figs. 20.1–5. 1958 (habit, flower, fruit). Figure 20.3 shows a petal, not a sepal (see description in Leandri, 1958: 125).

The chosen lectotype for *Wielandia platyrachis* bears the diagnosis in Baillon's handwriting, and "*Petalodiscus platyrachis* †", instead of "*Savia (Petalodiscus) platyrachis* H. Bn." as on the duplicates.

Baillon spelled the epithet three times "platyrachis" (1858: 572; 1861: 33) but only once "platyrachys" (1858: 40). The former spelling is therefore adopted here. The spelling "rachis" instead of "rhachis" is legitimate and commonly used in botany (Stearn, 1992: 478). The variant "platyrhachis" is therefore not admissible according to Art. 60.1 of the Code (Greuter et al., 2000).

The names *Savia platyrachis* and *S. pulchella* were first mentioned in print (Baillon, 1858: 572–573) as nomina nuda.

This species is very variable in leaf shape, size, and texture, which accounts for Leandri's (1934, 1958) description of a conspecific species and two varieties (see list of synonyms). The only notable morphological variation observed by us is that two of the specimens examined (*Du Petit Thouars s.n.* and *Perrier de la Bâthie 2091*, both P) have staminate flowers with considerably longer filaments (2 mm long) than in all other collections. The anthers are of normal size but are exserted from the flower and not crowded together.

Distribution and ecology. Madagascar; Sambirano, western and eastern floristic domain, to

eastern border of central domain. Wet and littoral forest, on laterite and alluvial soil over basalt and sandstone; 20–1120 m altitude. Figure 12.

IUCN Red List category. *Wielandia platyrachis* is a relatively common species, occurring throughout the eastern part of the island including several protected areas (48 collections seen). In view of the high rate of destruction of primary and even secondary vegetation in Madagascar, however, using IUCN (2001) criteria, we recommend that it be rated NT (Near Threatened).

Etymology. The epithet refers to the conspicuously flattened young twigs and inflorescence axes.

Vernacular names. Andromolahy (*s. coll. 59-R273*), Hazondomohina (*s. coll. 80-R118*), Ramy (*De la Croix 7411-RN*), Tsivoka (*SF 15260*).

Representative specimens examined. MADAGASCAR. **Antsiranana prov.:** Forêt d'Andrakaraha, près d'Antalaha, *Capuron 9228-SF* (K, MO, P); National Park of Montagne D'Ambre, path from station to Bianamalao, *Du Puy et al. MB214* (K, MO). **Toamasina prov.:** Ile de Nosy Mangabe, au fond de la baie d'Antongil, *Capuron 22897-SF* (K, MO, P); Colline de Menatany, au Sud de Soanierana-Ivongo, *Capuron 23828-SF* (K, MO, P); près d'Ankarahara (P.K. 100 de la route Tananarive–Moramanga), rive gauche du Mangoro, *Capuron 22908-SF* (K, MO, P). **Fianarantsoa prov.:** au P. K. 253 de la route Ivohibe–Farafangana près du Village de Beravy, *Capuron 23555-SF* (K, MO, P); Réserve Spéciale de Manombo, Parcelle 1, *Hoffmann et al. 284* (DAV, G, K, MO, P, TAN). **Toliara prov.:** Forêt de Bemangidy, au N de Mahatalaky (Ft. Dauphin), *Capuron 11793-SF* (K, MO, P); Ste. Luce, proposed QIT Madagascar Minerals ilmenite mining conservation area, *Hoffmann et al. 202* (K, MO, TAN); Environs de Ft. Dauphin, flanc E du pic St. Louis, *Humbert 5894* (A, P, US).

11. Wielandia ranavalonae (Leandri) Petra Hoffm. & McPherson, comb. nov. Basionym: *Savia ranavalonae* Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 239. 1957. TYPE: Madagascar. Tsinjoarivo, sur l'Onive, à l'Est d'Ambatolampy, 10 Apr. 1949, *R. Capuron (Service des Eaux et Forêts) 602* (lectotype, designated here, P!; duplicates, K!, MO!). Figures 3A–C; 4A, B; 6F, J; 7F; 8E; 9F; 14.

Shrub or small tree, 2–6 m high, up to 10 cm DBH; bark smooth, pinkish grey; branches glabrous, minutely scabrous only when very young, young slightly flattened, later terete. *Stipules* deltoid, acute, ca. 1 × 0.5 mm, glabrous, caducous; *petioles* 2–6 × 1–1.5 mm, adaxially channeled, glabrous; petiolar vascular bundles in cross section cylindrical; *leaf blades* obovate, more rarely elliptic to oblong, apically retuse to rounded, tip of midvein glandular, usually dark, basally acute to rounded, not or (especially basally) slightly revolute, with exmedial ultimate marginal venation, 2.5–6 × 1–3.5 cm, glabrous, coriaceous, green to greyish green on both sides, dull; midvein adaxially prominent; remaining venation adaxially prominent or not prominent; all veins abaxially prominent; anticlinal walls of leaf epidermis straight; stomata with (2 or)3(or 4) subsidiary cells. *Flowers* green with white petals and a yellowish disc when fresh. *Staminate flowers* cauline or axillary, solitary or in fascicles of up to 3 flowers, 2–3 × 4–5 mm, delicate; bracts deltoid, acute, ca. 0.7 × 0.3–0.5 mm, minutely scabrous; *pedicels* 2–5 × ca. 0.5 mm, terete or flattened, glabrous; *sepals* 5, erect, ca. 1.5–2 × 1.5–2 mm, nearly orbicular, rounded, glabrous, erose; the outermost smaller than the innermost; the innermost slightly clawed; *petals* 5, 2–3 × 1–1.5 mm, oblong, ± rounded, erose, glabrous; *disc* fleshy, annular but deeply lobed; lobes 5, 0.5–0.7 × ca. 1 mm, alternipetalous, distally truncate, erose or 2-lobed, erect, glabrous; *stamens* 5, 1–2 mm long; filaments ca. 1 × 0.2 mm, ± terete, fused for about half of their length; anthers 0.3–0.5 × 0.3–0.5 mm; connectives not thickened; thecae parallel; pistillode 0.2–0.5 × 0.2–0.5 mm, 2- or 3-lobed, glabrous. *Pistillate flowers* axillary, solitary, 4–5 × ca. 6 mm, robust; bracts deltoid, acute, ca. 1 × 1 mm, minutely scabrous; *pedicels* 10–20 × 0.5–1.5 mm, terete to flattened, distally slightly thickened, glabrous; *sepals* 5, erect, 1.5–2.5 × ca. 1.5 mm, ± membranous, fimbriate, otherwise glabrous; the outermost deltoid, acute to obtuse; the innermost oblong, rounded; *petals* 5, spathulate, orbicular, or rhombic, acute to rounded, clawed, ± erose, 3–4 × 1–2 mm, glabrous, about twice as long as sepals; *disc* annular, 5-lobed: lobes alternipetalous, ca. 1 mm long, distally ± 2-lobed, glabrous; *ovary* 3-locular, cylindrical to globose, ca. 1 mm long, glabrous; styles 3, 2-fid to the base; stylar branches short and thick, horizontally spreading to recurved, 0.5–0.8 mm long, basally ca. 0.5 mm thick, glabrous; stigmas acute. *Fruits* solitary, subglobose, 3-lobed, 8–15 × 10–16 mm, glabrous, reticulate to rugose, smooth, light yellowish brown to medium brown; fruiting pedicels (3–)10–20 × 1–1.5 mm, terete or flattened, glabrous; columella 8–12 × ca. 1 mm, basally thickened to 1.5 mm, distally clavate to 2–3 mm for 3–4 mm of its length; disc and perianth persistent; exocarp 0.2–0.3 mm thick; endocarp 0.3–0.5 mm thick; *seeds* 1 or 2 per locule; in 1-seeded locules ellipsoid, 7–10 × 7–10 × 5–6 mm, smooth, blackish brown, with large hilum (ca. 4 × 1 mm) in the center of the ventral face; micropyle lateral, directly above hilum, protruding ca. 1 mm; embryo ellipsoid with hemi-ellipsoid cotyledons; radicle subterminal, hardly longer than wide.

No collection number is given in the protologue of the basionym *Savia ranavalonae* (Leandri, 1957); however, the lectotype is the only specimen cited by

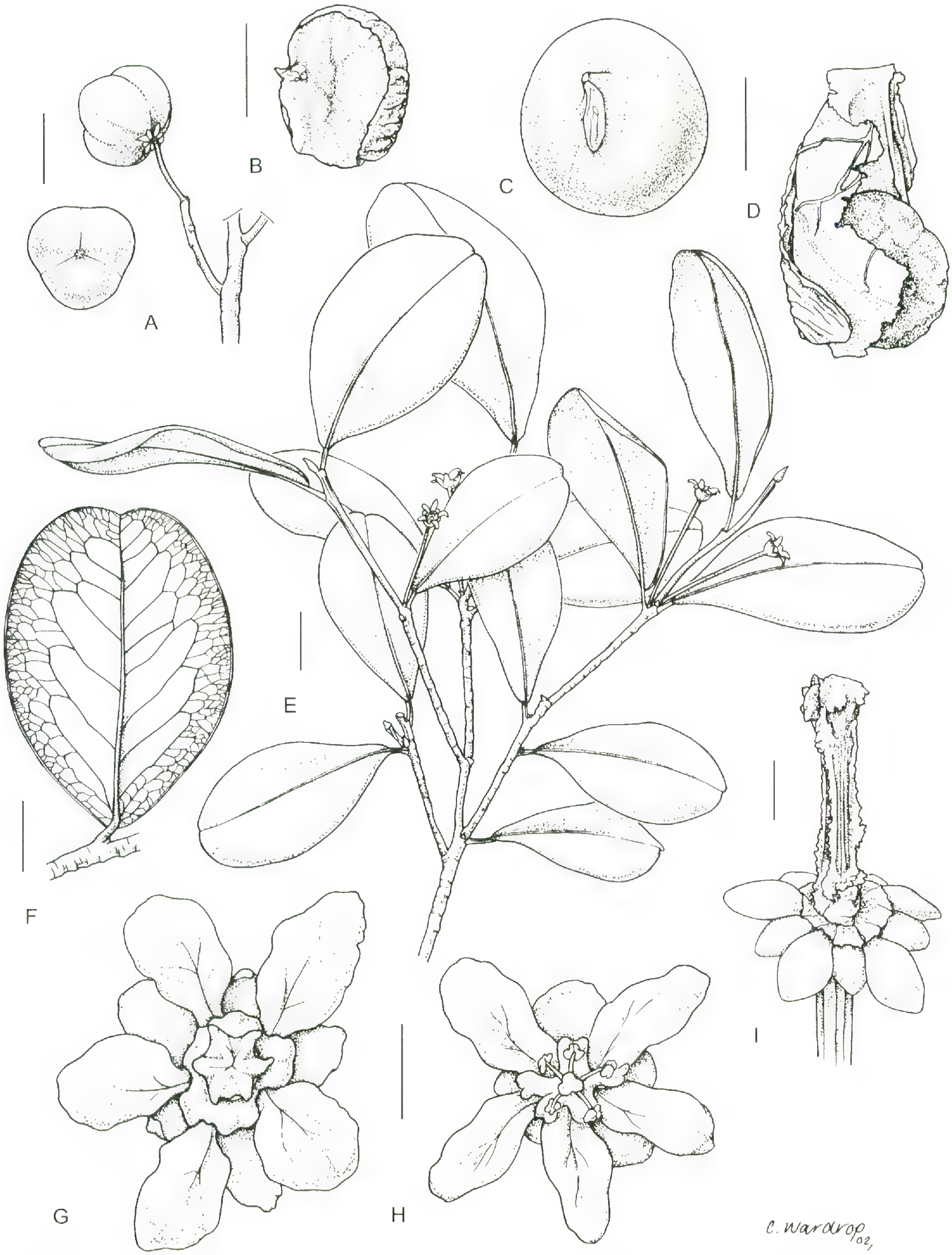

Figure 14. *Wielandia ranavalonae*. —A. Fruit, seen from above and lateral view. —B. Dry cotyledon with radicle. —C. Seed, ventral view. —D. Fruit fragment with dry seed. —E. Flowering branch. —F. Leaf. —G. Pistillate flower. —H. Staminate flower. —I. Columella with persistent perianth and disc. A, E, G–I, *Capuron 20586-SF* (K); B–D, *Perrier de la Bâthie 16954* (K); F, *Capuron 602-SF* (P). Scale bars = 10 mm in A, E, F; 5 mm in B–D; 2 mm in G–I. Drawn by Catherine Wardrop.

Leandri later in the *Flore* (1958: 122). No other specimen collected by Capuron before 1960 was deposited in Paris under this name.

Distribution and ecology. Western and central Madagascar and Comoro Islands (Mayotte). Dry and montane forest in Madagascar between 100 and 2000 m; in Mayotte in lowland rainforest between 90 and 120 m altitude. Figure 11.

IUCN Red List category. *Wielandia ranavalonae* has been collected only nine times in Madagascar and twice in Mayotte. However, its distributional range is very large, and it seems to have little preference as to altitude and vegetation type. The species is locally common in the single locality (an area of basaltic rock of less than 1 km^2) where it occurs in Mayotte (F. Barthelat, pers. comm.). It occurs in the protected areas of Andohahela and Zombitsy and, encouragingly, several recent collections bear fruit. We therefore suggest that, despite the few known collections, it be assigned to IUCN (2001) category NT (Near Threatened).

Etymology. This species is named after one of three Malagasy queens, Ramavo Ranavalona I (1790–1861), Ramona Ranavalona II (1829–1883), or Razafindrahety Ranavalona III (1861–1917).

Vernacular names. Mayotte: Tsatsikysembitry kely (Shibuchi; *Labat et al. 3290*).

Representative specimens examined. COMORO ISLANDS. Mayotte, Chiconi, Sohoa, réserve forestière de Sohoa, *Labat et al. 3290* (K, MO, P). MADAGASCAR. **Antsiranana prov.:** Daraina, forêt d'Antsahabe, *Nusbaumer 981* (G, K); **Toliara prov.:** Andohahela RN, *Eboroke et al. 946* (MO, P); Forêt d'Analavelona (bassin du Fiherenana), *Humbert 19760* (K, P); Zombitsy, Réserve Naturelle 7, PK 783, à 1 km au N du village d'Andranomaitso en dehors de la réserve, le long du layon qui limite la réserve, *Randrianaivo et al. 180* (MO).

12. Wielandia tanalorum (Leandri) Petra Hoffm. & McPherson, comb. nov. Basionym: *Blotia tanalorum* Leandri, Mém. Inst. Sci. Madagascar, Sér. B, Biol. Vég. 8: 243. 1957. TYPE: Madagascar. Farafangana prov.: Ivohibe, bord de chemin, *R. Decary 5104* (lectotype, designated by Hoffmann & McPherson, 1998: 259, P!; duplicate, K!).

IUCN Red List category. *Wielandia tanalorum* has been less frequently collected than other former *Blotia* species, but its occurrence over a large area in eastern Madagascar, running all the way from the north to the south of the island (Hoffmann & McPherson, 1998: fig. 4D), means that it is assigned the IUCN (2001) rating of NT (Near Threatened).

For description, a complete list of specimens studied, and a distribution map see Hoffmann and McPherson (1998).

Selected specimens examined. MADAGASCAR. **Antsiranana prov.:** Vallée intérieure de l'Androranga, affl. de la Bemarivo (nord-est) aux environs d'Antongodriha, Mont Anjenabe, *Humbert & Capuron 24041* (K, MO, P). **Toamasina prov.:** Farafangana, Ivohibe, *Decary 5104* (K, MO, P).

13. Wielandia unifex Petra Hoffm. & McPherson, sp. nov. TYPE: Madagascar. Antsiranana prov.: Sahafary, 15°17.41′S, 50°22.46′E, 39 m, 13 Oct. 2000, *R. Randrianaivo & R. Bernard 556* (holotype, K!; isotypes, MO!, TAN!, US!). Figure 11.

Haec species inter species generis *Wielandiae* combinatione apiculum foliorum longi-acuminatorum cum petiolis teretibus, longis (4–6 mm), gracilibus (0.5–0.8 mm diametro), petalis sepalis brevioribus, disco glabro distinguenda.

Treelet 3 m; indumentum absent; young twigs terete. *Stipules* mainly broken off, some basal portions remaining; to 1.2 mm long, ca. 0.7 mm wide, hard; *petioles* 4–6 × 0.5–0.8 mm, terete; *leaf blades* elliptic-oblong, sometimes slightly ovate or obovate, apically long-acuminate (acumen 10–20 mm, ultimately ± rounded), basally acute to obtuse, not decurrent, 4.5–6.5 × 1.5–2.3 cm, thickly chartaceous, drying olive-green and dull on both sides; venation slightly prominent on both sides, 5 to 7 secondary veins per leaf half, long, simple, unbranched intersecondary veins present in most intercostal fields, areoles very small and regular. *Flowers* not seen. *Infructescences* axillary, with fruits solitary or rarely in twos; fruiting pedicels (7–)10–15 mm long, terete, inarticulate; bracts deltoid, acute, 0.5–1 × 0.5 mm, entire, scarious; perianth and disc persistent in fruit; *sepals* 5, oblong, apically rounded, ca. 1.5 × 1–1.2 mm, entire; *petals* 5, broadly spathulate, apically rounded, ca. 1 × 0.6–0.7 mm, slightly erose, clawed at base to about a third of their length, shorter than sepals, apex reflexed in fruit; *disc* annular, glabrous, ca. 0.5 mm long, deeply alternipetalously 5-lobed; lobes apically truncate, ca. 0.3 × 0.7–0.8 mm. *Fruit* a 3-locular schizocarp, estimated ca. 9 mm in diameter (only dehisced mericarps seen), dehiscing septicidally, loculicidally, and septifragally; dehiscence of septae irregular; styles 3, bifid; stigmas acute; exocarp strongly reticulate, green drying olive-green, 0.1–0.2 mm thick, endocarp ca. 0.4 mm thick; columella 6–7 × 0.8–1 mm, basally hardly thickened, distally thickened to 1.5 mm, ca. 6× longer than the narrowest width; *seeds* immature, 1 or 2 per locule, reddish brown.

IUCN Red List category. *Wielandia unifex* is rated DD (Data Deficient), by IUCN (2001) criteria, because

it is only known from the type. The collection notes indicate that it is locally common.

Etymology. The epithet is Latin and means "the One-Maker." It refers to the crucial role that the discovery of this taxon played in our decision to unite all Western Indian Ocean taxa of tribe Wielandieae in one genus, *Wielandia*.

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APPENDIX 1. List of species.

1. *Wielandia bemarensis* (Leandri) Petra Hoffm. & McPherson
2. *Wielandia bojeriana* (Baill.) Petra Hoffm. & McPherson
3. *Wielandia danguyana* (Leandri) Petra Hoffm. & McPherson
4. *Wielandia elegans* Baill.
5. *Wielandia fadenii* (Radcl.-Sm.) Petra Hoffm. & McPherson
6. *Wielandia laureola* (Baill.) Petra Hoffm. & McPherson
7. *Wielandia leandriana* (Petra Hoffm. & McPherson) Petra Hoffm. & McPherson
8. *Wielandia mimosoides* (Baill.) Petra Hoffm. & McPherson
9. *Wielandia oblongifolia* (Baill.) Petra Hoffm. & McPherson
10. *Wielandia platyrachis* (Baill.) Petra Hoffm. & McPherson
11. *Wielandia ranavalonae* (Leandri) Petra Hoffm. & McPherson
12. *Wielandia tanalorum* (Leandri) Petra Hoffm. & McPherson
13. *Wielandia unifex* Petra Hoffm. & McPherson

PHYLOGENETIC ANALYSIS OF MORPHOLOGICAL CHARACTERS IN *OURISIA* (PLANTAGINACEAE): TAXONOMIC AND EVOLUTIONARY IMPLICATIONS[1]

Heidi M. Meudt[2,3] *and Beryl B. Simpson*[2]

Abstract

Ourisia Comm. ex Juss. (Plantaginaceae; Scrophulariaceae s.l.) is a genus of herbaceous or suffruticose species that occur largely in high-elevation habitats of Andean South America, New Zealand, and Tasmania. Recent molecular phylogenetic data have clarified evolutionary relationships within *Ourisia* and provided high support for the recognition of two subgenera based primarily on habit, i.e., *Ourisia* subg. *Ourisia* (25 herbaceous species) and *Ourisia* subg. *Suffruticosae* Meudt (three suffruticose species). To investigate the utility of morphological data in comparison to molecular data for phylogeny and subgeneric taxonomy, phylogenetic hypotheses were generated using 20 morphological characters for all 33 taxa of *Ourisia* using *Melosperma* Benth. as an outgroup. Additional phylogenetic hypotheses were generated by combining the morphological data with molecular data for the 29 species (plus *Melosperma*) for which the latter were available. With respect to phylogeny, morphological data are congruent with molecular data but generally provide less resolution. Morphological data are informative regarding infrageneric taxonomy and show that at least six morphological characters in addition to habit support the delimitation of the two subgenera. Finally, morphological characters of interest were traced onto the molecular phylogeny. Evolution of these and other characters are discussed in light of the various phylogenetic results.

Key words: Character evolution, infrageneric taxonomy, morphology, *Ourisia*, phylogeny, Plantaginaceae, Scrophulariaceae s.l.

Resumen

Ourisia Comm. ex Juss. (Plantaginaceae; Scrophulariaceae s.l.) es un género de especies herbáceas o sufruticosas que crecen predominantemente en hábitats de grandes altitudes de los Andes de Sudamérica, Nueva Zelandia y Tasmania. Datos filogenéticos recientes han aclarado las relaciones evolucionarias dentro de *Ourisia* y han dado un gran respaldo al reconocimiento de dos subgéneros basados principalmente en hábito, es decir, *Ourisia* subg. *Ourisia* (25 especies herbáceas) y *Ourisia* subg. *Suffruticosae* Meudt (tres especies sufruticosas). A fin de investigar la utilidad de los datos morfológicos en comparación con los datos moleculares de filogenia y taxonomía subgenérica, se generaron hipótesis filogenéticas usando 20 caracteres morfológicos para los 33 taxa de *Ourisia*, con *Melosperma* Benth. como grupo externo. Se generaron otras hipótesis filogenéticas combinando los datos morfológicos con los datos moleculares de las 29 especies (más *Melosperma*) en las que se disponía de estos últimos. Respecto de la filogenia, los datos morfológicos son coherentes con los datos moleculares, pero por lo general ofrecen menos resolución. Los datos morfológicos son informativos respecto de la taxonomía infragenérica e indican que al menos seis caracteres morfológicos, además del hábito, respaldan la delimitación de los dos subgéneros. Por último, se trazaron a la filogenia molecular caracteres que resultan de interés. Se analiza la evolución de estos y otros caracteres a la luz de los diversos resultados filogenéticos.

Species of *Ourisia* Comm. ex Juss. (Plantaginaceae; Scrophulariaceae s.l.) are distributed throughout Andean South America, New Zealand, and Tasmania, largely in high-elevation habitats up to 5000 m but extending down to sea level especially at high latitudes. Within *Ourisia*, 28 species are currently recognized (Meudt, 2006) and have five-lobed calyces, tubular to tubular-funnelform corolla tubes, five

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corolla lobes, four didynamous stamens adnate to the corolla tube (a staminode may or may not be present), and a bicarpellate pistil. Many of these floral traits are common in other genera of Scrophulariaceae s.l. (Kampny, 1995; Kampny & Dengler, 1997; Judd et al., 2002), which includes the recently re-circumscribed family Plantaginaceae to which *Ourisia* belongs (APG II, 2003; Albach et al., 2005).

Species of *Ourisia* also have opposite leaves, a capitate to emarginate stigma, and loculicidal capsules with many tiny, reticulate seeds. Species may be suffruticose with numerous leafy branches arising from a woody base, or herbaceous with repent or decumbent, branching stems. Flowers may be solitary or arranged in pairs or whorls in racemose, bracteate inflorescences. Species of *Ourisia* have red to purple pigments that may be found to various degrees on leaves, bracts, peduncles, pedicels, and/or calyces. Although the suite of floral and vegetative characters mentioned above may be used to distinguish *Ourisia* from related genera, none of the characters by themselves are unique to, or diagnostic for, the genus.

Phylogenetic analyses based on chloroplast and nuclear DNA sequence data have recently been performed to elucidate the biogeography and relationships among species of *Ourisia* (Meudt & Simpson, 2006). One interesting result from these analyses was high support for two clades within the genus that can be distinguished morphologically simply by habit. This, in part, led to the description of two subgenera within *Ourisia*: *Ourisia* subg. *Ourisia*, comprising all 25 herbaceous species, and *Ourisia* subg. *Suffruticosae* Meudt, with the three suffruticose species (Meudt, 2006). This infrageneric taxonomy contrasts with previous treatments such as that of Poeppig and Endlicher (1835; Table 1) followed by most subsequent workers, in which *Ourisia* subg. *Ourisia* consisted of species with irregular calyces and *Ourisia* subg. *Dichroma* (Cav.) Poepp. & Endl. of species with regular calyces.

The main goals of the present study were to analyze an array of morphological characters to: (1) investigate their significance with respect to the phylogeny and subgeneric taxonomy of *Ourisia*, and (2) understand patterns of evolution within *Ourisia* for specific morphological characters. To this end, a phylogenetic approach was employed, in which novel morphological data and previously published molecular phylogenetic data from Meudt and Simpson (2006) were analyzed separately and together. Morphological characters of interest were also optimized onto one of the molecular phylogenetic trees in order to assess their evolution within *Ourisia*.

Materials and Methods

Taxa included

Ingroup. Morphological data were collected from all 33 taxa (28 species) in *Ourisia*. Previously published molecular data (Meudt & Simpson, 2006) were available for only 29 taxa (26 species) because high-quality DNA could not be extracted from four taxa (i.e., *O. breviflora* Benth. subsp. *uniflora* (Phil.) Meudt, *O. biflora* Wedd., *O. coccinea* (Cav.) Pers. subsp. *coccinea*, *O. fuegiana* Skottsb.).

Outgroup. Historically, *Ourisia* has been placed in tribe Digitalideae or Veroniceae (see Meudt, 2006 for an overview), but a recent molecular phylogenetic study of Plantaginaceae has shown that these tribes are not monophyletic and *Ourisia* is not closely related to any of the genera from these tribes (Albach et al., 2005). Instead, *Ourisia* was found to be part of a clade that contains at least 10 other genera from the tribes Gratioleae (*Bacopa* Aubl., *Gratiola* L., *Mecardonia* Ruiz & Pav., *Otacanthus* Lindl., *Scoparia* L., and *Stemodia* L.), Angelonieae (*Angelonia* Humb. & Bonpl. and *Basistemon* Turcz.), and Melospermeae (*Melosperma* Benth. and *Monttea* Gay) (Albach et al., 2005). In their combined analysis of four DNA markers (Albach et al., 2005), *Ourisia* was sister to Angelonieae–Melospermeae but with very little support (< 50 bootstrap percentage [BP]; 0.88 posterior probability [PP]); Gratioleae in turn was monophyletic and sister to *Ourisia* and Angelonieae–Melospermeae. Of the four genera in this latter group, *Melosperma* is morphologically most similar to *Ourisia* (especially the suffruticose species) in its basally woody and creeping (to subshrub) habit, sessile leaves, presence of hypogynous disc, and solitary, axillary flowers. The presence of these characters allows more comparable scoring of morphological characters than any other genus. In addition, unlike *Angelonia*, *Monttea*, and some species of *Basistemon*, the flowers of *Melosperma* and *Ourisia* do not produce oil as a reward for pollinators. Finally, *Melosperma* is a monotypic genus endemic to the southern Andes, where *Ourisia* is postulated to have arisen (Meudt & Simpson, 2006). For these reasons, *Melosperma* appears to be the best choice for the outgroup until all genera in the family (and especially in Gratioleae) are included in a phylogenetic analysis and a sister group to *Ourisia* is elucidated with high support. A thorough analysis and discussion of character evolution including more genera from the clade to which *Ourisia* belongs, and indeed from Plantaginaceae as a whole, are clearly warranted but are beyond the scope of this study.

Table 1 lists the geographical area of distribution, habit, authorities, representative voucher information,

Table 1. Taxa sampled for this study, including geographical area, habit, authorities, voucher information, and subgeneric classification.

Geographical area and habit	Species	Representative voucher	Subgeneric treatment: Poeppig & Endlicher, 1835	Subgeneric treatment: Meudt, 2006
South America				
North-central Andean herbaceous taxa				
	Ourisia biflora Wedd.	Bolivia, La Paz, *Mandon s.n.* (P); Peru, Puno, *Stafford 1112* (F)	*Ourisia*	*Ourisia*
	Ourisia chamaedrifolia Benth.	Peru, Cusco, *Meudt & López 052* (TEX); Venezuela, Mérida, *Dorr & Barnett 5228* (NY)	*Dichroma*	*Ourisia*
	Ourisia cotapatensis Meudt & S. Beck	Bolivia, La Paz, *Beck 22777* (LPB); Bolivia, La Paz, *Beck 25989* (LPB)	*Dichroma*	*Ourisia*
	Ourisia muscosa Benth.	Colombia, Caldas, *Rangel 1816* (NY); Peru, Ancash, *Refulio et al. 23* (TEX)	*Dichroma*	*Ourisia*
	Ourisia pulchella Wedd.	Bolivia, Cochabamba, *Brooke 6271* (BM); Bolivia, La Paz, *Kessler 3397* (TEX)	*Ourisia*	*Ourisia*
Southern Andean herbaceous taxa				
	Ourisia alpina Poepp. & Endl.	Argentina, Neuquén, *Rossow 1367* (NA); Chile, IX Región, *Meudt et al. 040* (TEX)	*Dichroma*	*Ourisia*
	Ourisia breviflora Benth. subsp. *breviflora*	Argentina, Tierra del Fuego, *Meudt & López 058* (TEX); Chile, XI Región, *Donat s.n.* (PH)	*Dichroma*	*Ourisia*
	Ourisia breviflora Benth. subsp. *uniflora* (Phil.) Meudt	Argentina, Río Negro, *Boelcke & Correa 5583* (SI); Chile, VIII Región, *Werderman 1348* (F)	*Dichroma*	*Ourisia*
	Ourisia coccinea (Cav.) Pers. subsp. *coccinea*	Argentina, Neuquén, *Rossow 2091* (NA); Chile, IX Región, *Montero 8508* (OS)	*Dichroma*	*Ourisia*
	Ourisia coccinea (Cav.) Pers. subsp. *elegans* (Phil.) Meudt	Chile, VIII Región, *Meudt & López 007* (TEX); Chile, X Región, *Hollermayer 322* (UC)	*Dichroma*	*Ourisia*
	Ourisia fragrans Phil.	Argentina, Río Negro, *Meudt & López 056* (TEX); Chile, X Región, *Sparre & Constance 10832* (UC)	*Dichroma*	*Ourisia*
	Ourisia fuegiana Skottsb.	Argentina, Tierra del Fuego, *Goodall 3437* (NA); Chile, IX Región, *Hicken-Reichert s.n.* (SI)	*Dichroma*	*Ourisia*
	Ourisia pygmaea Phil.	Argentina, Neuquén, *Correa 5711* (BAB); Chile, IX Región, *Meudt et al. 041* (TEX)	*Dichroma*	*Ourisia*
	Ourisia ruellioides (L. f.) Kuntze	Argentina, Tierra del Fuego, *Meudt & López 059* (TEX); Chile, VII Región, *Meudt & López 033* (TEX)	*Ourisia*	*Ourisia*
Southern Andean suffruticose taxa				
	Ourisia microphylla Poepp. & Endl.	Argentina, Neuquén, *Gentilli 212* (BAB); Chile, VII Región, *Meudt & López 036* (TEX)	*Dichroma*	*Suffruticosae*

Table 1. Continued.

Geographical area and habit	Species	Representative voucher	Subgeneric treatment: Poeppig & Endlicher, 1835	Subgeneric treatment: Meudt, 2006
	Ourisia polyantha Poepp. & Endl.	Chile, Región Metropolitana, *Gradjot 3464* (GH); Chile, VI Región, *Arroyo 201149* (TEX)	*Dichroma*	*Suffruticosae*
	Ourisia serpyllifolia Benth.	Chile, IV Región, *Morrison 17045* (UC); Chile, IX Región, *Meudt & López 035* (TEX)	*Dichroma*	*Suffruticosae*
Australia				
Tasmanian herbaceous taxa				
	Ourisia integrifolia R. Br.	Australia, Tasmania, *Collier 1117* (HO); Australia, Tasmania, *Meudt et al. 065* (TEX)	*Dichroma*	*Ourisia*
New Zealand				
New Zealand herbaceous taxa				
	Ourisia caespitosa Hook. f.	New Zealand, North Island, *Druce s.n.* (CHR); New Zealand, South Island, *Meudt & López 91* (TEX)	*Ourisia*	*Ourisia*
	Ourisia confertifolia Arroyo	New Zealand, South Island, *Dickinson et al. s.n.* (OTA); New Zealand, South Island, *Meudt & López 093* (TEX)	*Ourisia*	*Ourisia*
	Ourisia crosbyi Cockayne	New Zealand, South Island, *Meudt & López 089* (TEX); New Zealand, Stewart Island, *Petrie s.n.* (AK)	*Dichroma*	*Ourisia*
	Ourisia glandulosa Hook. f.	New Zealand, South Island, *Adams s.n.* (WELT); New Zealand, South Island, *Meudt & López 090* (TEX)	*Ourisia*	*Ourisia*
	Ourisia macrocarpa Hook. f. subsp. *calycina* (Colenso) Arroyo	New Zealand, South Island, *Meudt & López 085* (TEX); New Zealand, South Island, *Obrien 90/1* (AK)	*Dichroma*	*Ourisia*
	Ourisia macrocarpa Hook. f. subsp. *macrocarpa*	New Zealand, South Island, *Meudt & López 097* (TEX); New Zealand, South Island, *Petrie s.n.* (WELT)	*Ourisia*	*Ourisia*
	Ourisia macrophylla Hook. subsp. *lactea* (L. B. Moore) Meudt	New Zealand, South Island, *Kalin 675* (CANU); New Zealand, South Island, *Meudt & López 080* (TEX)	*Dichroma*	*Ourisia*
	Ourisia macrophylla Hook. subsp. *macrophylla*	New Zealand, North Island, *Meudt & López 067* (TEX); New Zealand, North Island, *Meudt & López 076* (TEX)	*Dichroma*	*Ourisia*
	Ourisia modesta Diels	New Zealand, South Island, *Druce s.n.* (CHR); New Zealand, Stewart Island, *Moore s.n.* (CHR)	*Ourisia*	*Ourisia*
	Ourisia remotifolia Arroyo	New Zealand, South Island, *Arroyo 73153* (CHR); New Zealand, South Island, *Meudt & López 094* (TEX)	*Ourisia*	*Ourisia*
	Ourisia sessilifolia Hook. f. subsp. *sessilifolia*	New Zealand, South Island, *Meudt & López 096* (TEX); New Zealand, Stewart Island, *Oliver 5* (WELT)	*Dichroma*	*Ourisia*

Table 1. Continued.

Species	Geographical area and habit	Representative voucher	Subgeneric treatment	
			Poeppig & Endlicher, 1835	Meudt, 2006
Ourisia sessilifolia Hook. f. subsp. *splendida* (L. B. Moore) Arroyo	New Zealand, South Island, Mt. Arthur	*Cockayne s.n.* (WELT); New Zealand, South Island. *Meudt & López 102* (TEX)	*Dichroma*	*Ourisia*
Ourisia simpsonii (L. B. Moore) Arroyo	New Zealand, South Island, Mt. Arthur	*Druce s.n.* (CHR); New Zealand, South Island. *Meudt & López 081* (TEX)	*Ourisia*	*Ourisia*
Ourisia spathulata Arroyo	New Zealand, South Island, Mt. Arthur	*Mark s.n.* (OTA); New Zealand, South Island. *Meudt & López 100* (TEX)	*Ourisia*	*Ourisia*
Ourisia vulcanica L. B. Moore	New Zealand, North Island, Mt. Ruapehu	*Buchanan 13513* (HO); New Zealand, North Island. *Meudt & López 071* (TEX)	*Ourisia*	*Ourisia*
Outgroup				
Melosperma andicola Benth.	Chile, Región Metropolitana, Cordillera Arrayán	*Arroyo & Humaña 206607* (TEX)	*Melosperma*	

and subgeneric classification of all ingroup and outgroup taxa.

MOLECULAR DATA

The materials and methods for DNA extraction, amplification via polymerase chain reaction, cloning, sequencing, and alignment of sequences are described in detail in Meudt and Simpson (2006). The four markers sequenced were: the nuclear ribosomal DNA (nrDNA) internal transcribed spacer region (ITS; including ITS-1, 5.8S, and ITS-2) and part of the external transcribed spacer region (ETS), as well as two chloroplast DNA (cpDNA) regions (*matK* 3′ intron and *rps16* intron). All sequences from Meudt and Simpson (2006) were previously submitted to GenBank. Because tree topologies for the four individual molecular markers were largely congruent (Meudt & Simpson, 2006), they were combined into one matrix and treated as a single molecular data set for all analyses.

MORPHOLOGICAL DATA

Morphological data were scored from a total of 347 herbarium specimens, including two to 44 per species (median = 10, mean = 12.7), depending on geographical range, variability, and availability of specimens (Meudt, 2006). Due to the tendency of specimens to turn black upon drying, color data were only rarely obtained from the specimen itself and information supplied on the collection labels was frequently used. A newly relevant character for the genus, seed coat morphology, was added by scanning electron microscopy (SEM) of seeds (Meudt, 2006). Chromosome counts were taken from the literature (see Meudt, 2006: appendix 2). Morphological characters for *Melosperma andicola* Benth. were scored using descriptions from the literature (Rossow, 1985; Sérsic & Cocucci, 1999). Of the many characters that were studied, 20 were chosen for phylogenetic analysis based on general ease of scoring, ability to discern discrete, non-overlapping character states, and interest in tracing the evolution of that particular character. The resulting morphological data matrix of 20 characters for all 33 ingroup taxa plus *Melosperma* is given in Table 2; a detailed list of these characters and their character states is found in Appendix 1.

Twelve of the 20 morphological characters were variable in at least one taxon. Kornet and Turner (1999) reviewed and compared the seven different ways to code polymorphisms for phylogeny reconstruction. We followed their suggestion that, when the ancestral state is unknown, the preferred method is to

Table 2. Morphological characters included in this study and the character states for each species of *Ourisia* and the outgroup *Melosperma andicola*. Asterisks (*) indicate the four taxa that were not included in the molecular analyses and were thus deleted from the morphological matrix before combining the molecular and morphological data sets. Characters and character states are described in Appendix 1.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Melosperma andicola	0	0	–	0	0	–	0	2	01	0	1	0	0	0	–	01	–	0	–	–
Ourisia alpina	1	1	0	1	1	23	1	01	01	0	0	0	0	0	1	01	01	1	1	–
*Ourisia biflora**	1	1	0	01	1	1	0	0	01	1	0	0	1	0	0	1	0	–	–	–
Ourisia breviflora subsp. *breviflora*	1	1	0	01	1	12	1	01	01	0	0	0	0	1	2	1	01	1	1	–
Ourisia breviflora subsp. *uniflora**	1	1	0	01	1	1	1	0	1	0	0	0	0	0	12	01	01	1	1	–
Ourisia caespitosa	1	1	0	01	1	12	0	0	0	0	1	2	0	0	23	12	0	1	2	2
Ourisia chamaedrifolia	1	1	0	01	1	12	2	01	2	0	1	0	01	0	2	12	01	1	1	–
Ourisia coccinea subsp. *coccinea**	1	1	0	1	1	2	1	01	2	0	0	0	0	1	1	0	01	1	1	0
Ourisia coccinea subsp. *elegans*	1	1	0	1	1	23	1	01	2	0	0	0	0	1	1	0	01	1	1	–
Ourisia confertifolia	1	1	1	01	1	12	0	02	0	0	1	0	0	0	01	01	0	1	2	2
Ourisia cotapatensis	1	1	0	0	1	1	1	0	1	0	12	1	1	0	1	1	01	1	–	–
Ourisia crosbyi	1	1	0	2	1	23	1	0	0	0	1	2	0	0	01	01	0	1	2	2
Ourisia fragrans	1	1	0	01	1	2	2	0	01	1	0	0	0	0	0	2	0	1	1	–
*Ourisia fuegiana**	1	1	0	0	1	0	01	0	0	0	0	0	0	1	2	1	0	1	1	–
Ourisia glandulosa	1	1	1	01	1	12	0	0	0	0	12	0	0	0	01	01	0	1	2	2
Ourisia integrifolia	1	1	0	01	1	12	1	0	0	01	1	0	0	0	02	1	01	1	1	1
Ourisia macrocarpa subsp. *calycina*	1	1	0	1	1	3	1	0	0	0	1	2	01	0	01	1	0	1	2	2
Ourisia macrocarpa subsp. *macrocarpa*	1	1	0	1	1	3	0	0	0	0	1	2	0	0	01	01	0	1	2	2
Ourisia macrophylla subsp. *lactea*	1	1	0	1	1	3	1	02	0	0	1	2	1	0	1	01	0	1	2	2
Ourisia macrophylla subsp. *macrophylla*	1	1	0	1	1	23	1	0	0	0	1	2	1	0	1	01	0	1	2	2
Ourisia microphylla	0	0	–	0	0	–	1	2	01	1	0	0	0	0	0123	01	2	0	0	01
Ourisia modesta	1	1	0	0	1	1	0	01	0	0	–	1	1	1	0	01	0	1	3	2
Ourisia muscosa	1	1	0	0	1	0	02	01	0	1	1	3	1	0	02	01	01	1	4	–
Ourisia polyantha	0	0	–	0	0	–	1	2	2	1	1	0	0	0	3	01	2	0	0	–
Ourisia pulchella	1	1	0	01	1	1	0	01	01	0	0	0	1	0	1	–	–	–	–	–
Ourisia pygmaea	1	1	0	0	1	0	1	0	01	0	0	0	0	0	01	1	0	1	1	–
Ourisia remotifolia	1	1	0	1	1	12	0	02	0	0	2	2	0	0	0	01	0	1	2	2
Ourisia ruellioides	1	1	0	2	1	12	0	01	2	1	1	0	0	1	02	1	01	1	1	0
Ourisia serpyllifolia	0	0	–	0	0	–	1	2	1	1	1	0	01	0	1	01	2	0	0	–
Ourisia sessilifolia subsp. *sessilifolia*	1	1	1	1	1	23	01	01	0	0	2	2	0	0	0	0	0	1	2	2
Ourisia sessilifolia subsp. *splendida*	1	1	1	1	1	2	1	0	0	0	2	1	0	0	0	1	0	1	2	2
Ourisia simpsonii	1	1	1	1	1	12	0	0	0	0	12	0	0	0	0	1	0	1	2	2
Ourisia spathulata	1	1	1	01	1	12	0	01	0	0	1	0	0	0	01	1	0	1	2	2
Ourisia vulcanica	1	1	0	1	1	23	0	0	0	0	1	2	1	0	12	01	0	1	2	2

code polymorphisms as ambiguous. This was accomplished by allowing variable taxa to contain multiple states (e.g., a taxon that was variable for a presence/absence of a character was scored using the notation 01; see Appendix 1) and setting MSTAXA = polymorphic for analyses in PAUP* v4.0b10 (Swofford, 2002) and MacClade v4.05 (Maddison & Maddison, 2000).

To avoid introducing bias into the data set, we treated all morphological characters as unordered and used reductive coding for most characters (Wilkinson, 1995). However, we chose a "composite" approach for scoring six characters (four regarding vestiture, i.e., characters 8, 12, 15, and 17, plus calyx symmetry [character 7] and staminode [character 16]). For these six characters, there were three approaches to scoring: (1) considering each a single, multistate character with "absent" as one of the character states; (2) assuming two independent characters, A (presence/absence) and B (type of structure), and scoring taxa that are variable for the presence/absence character as 01 in character A and as missing in character B; and (3) assuming two independent characters, A (presence/absence) and B (type of structure), and scoring taxa that are variable for the presence/absence character as 01 in character A and as the present state (e.g., state 1 or state 2) in character B.

Method 1 was chosen for these characters because many taxa were variable for presence/absence of these structures, and this method seemed the most straightforward coding method that required no further assumptions regarding variable taxa. For the four vestiture characters in particular, this resulted in "glabrous" (i.e., trichomes absent) being considered

a valid character state for vestiture, which we believe is a better choice than coding it as a separate, independent character. In general, trichomes in species of *Ourisia* may be found in varying densities on any given surface, from completely absent to forming a dense covering, suggesting that glabrous is a valid state at one end of the vestiture spectrum. In addition to the high occurrence of variability for presence/absence of trichomes in the data set, for two of the vestiture characters (8 and 17), taxa that had sessile glandular trichomes were always variable, 01. The staminode character 16 shows a similar pattern. This covariation (although not strict) suggests that the presence/absence and type "characters" may be biologically linked and should be treated as one character (Wilkinson, 1995). Method 1 also avoided creating extra characters with the majority of taxa scored as missing (i.e., ?), which can result in incorrect character optimization (Maddison, 1993; Strong & Lipscomb, 1999) or loss of information, or both. For example, two vestiture characters were constant when scored via method 2 because the majority of the taxa (30 and 31, respectively, out of 34 total) were scored as missing due to inapplicability or variability, making them phylogenetically uninformative and also very difficult to assess when mapped onto a molecular phylogeny.

For the reasons cited above and for consistency, we thus used the composite method 1 for scoring all four vestiture characters and for characters 7 and 16. Of course, as with reductive coding, composite coding has its deficiencies, especially when secondary losses occur (Strong & Lipscomb, 1999). In any case, choice of scoring method for these six characters does not appear to influence the phylogenetic analysis very much, as preliminary parsimony analyses using all three scoring options for these characters showed that there were only minor differences in tree topologies, tree scores, and bootstrap support for each of these methods (data not shown). Further empirical and simulation studies are needed, as the effect of these three coding options on the resulting phylogeny when taxa are variable for presence/absence does not appear to have been discussed in the literature regarding reductive versus composite coding (e.g., Maddison, 1993; Wilkinson, 1995; Strong & Lipscomb, 1999).

Two morphological matrices were assembled: one with all 33 ingroup taxa plus the outgroup, and a second with only the 29 ingroup taxa sampled for the molecular phylogeny, plus the outgroup. A combined data set was then assembled by adding this latter morphological matrix to the molecular data from Meudt and Simpson (2006) for 29 ingroup taxa (representing 26 species), plus *Melosperma*.

PHYLOGENETIC ANALYSES

The incongruence length difference (ILD) test (Farris et al., 1995) was used to test for significant heterogeneity of the molecular versus the morphological data sets by running 1000 permutations of the partition homogeneity test with MAXTREES set to 1000 for each comparison in PAUP*. The g1 statistic (Hillis & Huelsenbeck, 1992) was calculated in PAUP* as a measure of phylogenetic signal. Parsimony analyses were performed on all data sets in PAUP* with a simple heuristic search strategy, tree bisection-reconnection (TBR) branch swapping, 10 random addition replicates, MAXTREES set to 50,000, and gaps treated as missing data. One thousand bootstrap replicates were run with 10 random addition replicates and MAXTREES set to 1000. Bayesian analyses and one repetition of the maximum likelihood (ML) ratchet (Vos, 2003) were performed on the molecular data only using MrBayes v3.0b4 (Huelsenbeck & Ronquist, 2001) and PAUP*, respectively. The best model of evolution was chosen for the Bayesian and ML ratchet analyses using ModelTest v3.4 (Posada & Crandall, 1998). Because several most parsimonious trees (MPTs) resulted from the parsimony analysis of molecular data, and because these trees in turn were similar but not identical to trees from the Bayesian and likelihood ratchet analyses, one of these trees had to be chosen for optimization of morphological characters using MacClade. To this end, all resulting MPTs were scored via likelihood in PAUP* (using the best model of evolution chosen by ModelTest). Then the MPTs with the best ML score were compared to the Bayesian 50% majority rule tree and the likelihood ratchet best tree using topology and ML score as criteria for choosing one of the trees (see Results). For character optimization, the resolving option "show all most parsimonious states at each node" was used in MacClade.

Results

PHYLOGENETIC ANALYSES OF MOLECULAR DATA

The molecular data set of 29 ingroup taxa plus *Melosperma* contained significant phylogenetic signal according to the g1 statistic (Table 3). Parsimony analysis resulted in 288 MPTs of length 1066 (Table 3; trees not shown). Replicate Bayesian analyses using the model HKY + G + I resulted in a 50% majority rule tree that was fully resolved except for four polytomies where branches had < 0.50 PP. One of the resolutions of this tree is shown in Figure 1, which has a similar but not identical topology to some of the MPTs from the parsimony

Table 3. Comparison of parsimony phylogenetic analysis statistics for the *Ourisia* molecular and morphological data sets analyzed for this study. CI, consistency index; RI, retention index; RC, rescaled consistency index.

Data set	Total no. of terminals	No. of aligned characters	g1 Test*	No. of MPTs	Tree length	No. of parsimony informative characters (%)	CI	RI	RC
Molecular only (4 DNA regions combined)	30	2689	−2.135491	288	1066	375 (13.9%)	0.820	0.835	0.685
Morphological only	34	20	−0.558530	19,840	170	20 (100%)	0.818	0.756	0.618
Morphological only†	30	20	−0.747964	> 50,000	158	20 (100%)	0.823	0.731	0.601
Combined (molecular plus morphological)	30	2709	−2.129626	9	1372	395 (14.6%)	0.832	0.819	0.681

* g1 Test critical value = −0.09.
† Data set for the morphological matrix reduced to the 29 ingroup taxa plus *Melosperma*.

analysis. Trees from both analyses contained many of the same monophyletic groups of taxa, which in general were moderately to highly supported by parsimony BP values > 70% and PP values > 0.95 (BP and PP values are both shown on the Bayesian tree in Fig. 1). In these analyses, the three southern Andean suffruticose species *Ourisia microphylla* Poepp. & Endl., *O. polyantha* Poepp. & Endl., and *O. serpyllifolia* Benth. of *Ourisia* subg. *Suffruticosae* are highly supported as monophyletic (100 BP/1.0 PP) and sister to the herbaceous species of *Ourisia* subg. *Ourisia* (100 BP/1.0 PP), consistent with previous results (Meudt & Simpson, 2006).

Eighteen of the 288 MPTs composed two islands of trees that had the two best likelihood scores (−9644.32959 and −9644.65012, respectively). These MPTs were also the most similar to the Bayesian tree (Fig. 1; Bayesian tree scores range from −9676.07 to −9675.85) and the best tree from the ML ratchet (tree score −9640.93321, not shown). The main differences between these MPTs and trees from the Bayesian and ML ratchet are the relationships of some New Zealand species and the placement of Tasmanian *Ourisia integrifolia* R. Br. But, as was shown previously via parametric bootstrap, *O. integrifolia* cannot be rejected as the sister to the New Zealand clade (Meudt & Simpson, 2006). The Bayesian and likelihood ratchet trees therefore have topologies that appear to be representative of the evolutionary history of the genus. In addition, the best ML ratchet tree had a better ML score (−9640.93321) than all of the MPTs. Because the resolved Bayesian tree topology represents one of the possible resolutions of the polytomies in the best ML ratchet tree, this tree topology (Fig. 1) was chosen for use in character optimization.

PHYLOGENETIC ANALYSES OF MORPHOLOGY

The morphological data set contained phylogenetic signal according to the g1 statistic (Table 3). Parsimony analysis of the 20 morphological characters with all 33 ingroup taxa plus *Melosperma* resulted in 19,840 MPTs of length 170 (Table 3). The semi-strict consensus (or combinable component) tree of the MPTs (not shown; supplementary figure available from the author, as PDF online) shows all non-conflicting relationships and is a better representation of the data than the strict consensus tree due to the many MPTs with polytomies. In general, there is not much resolution in the semi-strict tree, but as with the analysis of molecular data (Fig. 1), there is (weak) support for *Ourisia* subg. *Suffruticosae* (61 BP). There is also high support for *Ourisia* subg. *Ourisia* (92 BP) and the sister relationship of *O. coccinea* subsp.

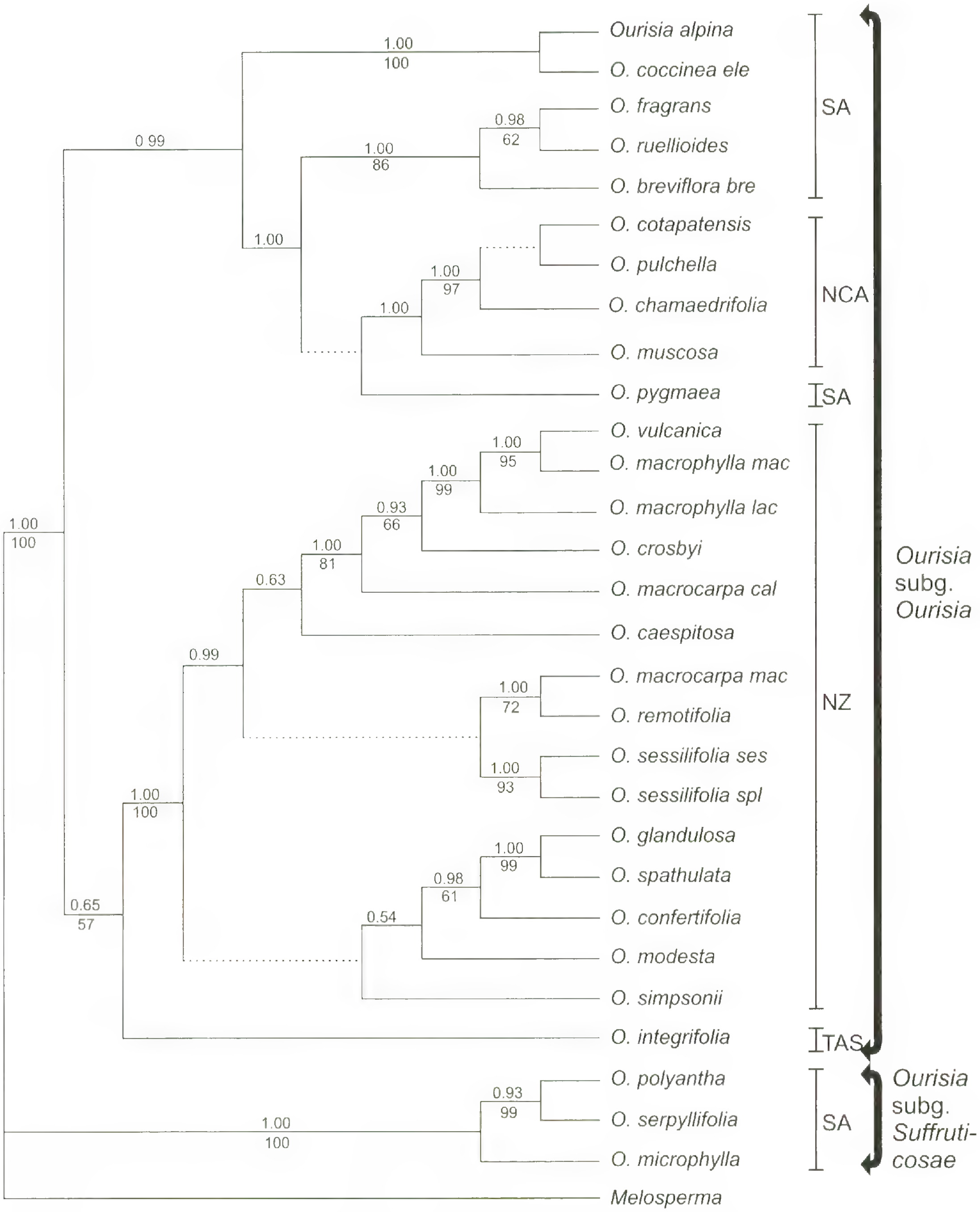

Figure 1. Bayesian phylogeny of the *Ourisia* molecular data set. One of the resolutions of the 50% majority rule consensus tree (which contained four branches with < 0.50 PP) of post-burn-in trees from one of the four replicates conducted for four million generations. This tree topology also represents one of the possible resolutions of the ML ratchet tree that had the highest ML score. Numbers above the branches indicate PP values, and numbers below the branches indicate BP values > 50 from the parsimony analysis of the same data set. The four branches with < 0.50 PP (dashed lines) collapse in the 50% majority rule tree. Brackets and letters to the right of taxon names refer to geographic area (SA, southern Andes; NCA, north-central Andes; NZ, New Zealand; TAS, Tasmania); thickened lines show subgeneric classification. Subspecies abbreviations are as follows: O. coccinea ele, *O. coccinea* subsp. *elegans*; O. breviflora bre, *O. breviflora* subsp. *breviflora*; O. macrophylla mac, *O. macropylla* subsp. *macrophylla*; O. macrophylla lac, *O. macrophylla* subsp. *lactea*; O. macrocarpa cal, *O. macrocarpa* subsp. *calycina*; O. macrocarpa mac, *O. macrocarpa* subsp. *macrocarpa*; O. sessilifolia ses, *O. sessilifolia* subsp. *sessilifolia*; O. sessilifolia spl, *O. sessilifolia* subsp. *splendida*.

elegans (Phil.) Meudt and *O. coccinea* subsp. *coccinea* (82 BP). This latter taxon was one of four that was unable to be sequenced; the other three (*Ourisia biflora*, *O. breviflora* subsp. *uniflora*, and *O. fuegiana*) were unresolved in the herbaceous clade. Only two other branches representing relationships among some of the New Zealand species are resolved in this tree (not shown), but they receive < 50 BP. This general lack of support in morphologically generated phylogenetic hypotheses is not uncommon and is likely due to the small number of characters analyzed (Bremer et al., 1999). Reducing the morphological matrix to the 29 ingroup taxa plus *Melosperma* gave > 50,000 MPTs of length 158 (Table 3). The topologies of the semi-strict consensus tree of this analysis were similar to but slightly less resolved than that of the analysis with all 34 taxa included (not shown).

PHYLOGENETIC ANALYSES OF MOLECULAR AND MORPHOLOGICAL DATA COMBINED

The ILD test found significant heterogeneity for the pairwise comparison of the morphological data set with the molecular data ($P < 0.001$). Visual inspection of the topologies of the Bayesian tree (Fig. 1) and the semi-strict consensus tree of the parsimony analysis of morphological data (not shown) showed that they do not conflict with one another. Indeed, even tree topologies that are very similar can still show a significant result in the ILD test (Johnson & Soltis, 1998), and here this might be due to the large difference in number of characters between the two data sets (2689 vs. 20). As the effect of the addition of morphological data on the phylogeny of *Ourisia* was of interest, the molecular and morphological data sets were combined into one data matrix despite the heterogeneity found in the ILD test. This combined matrix, like the individual data sets that compose it, contained phylogenetic signal (Table 3) and resulted in nine MPTs of 1372 steps each (Table 3; the strict consensus tree is shown in Fig. 2 and is identical to the semi-strict tree). These trees were generally similar topologically to those of the molecular-only analysis and contained clades with similar BP values (compare Figs. 1 and 2). However, the combined trees did differ topologically with most analyses of molecular data alone (Bayesian, ML ratchet, and MPTs with the highest ML) by the placement of *O. muscosa* Benth. as the sister to all other herbaceous species of *Ourisia*. Of the clades that received > 50 BP in the combined analysis, seven had the same value (including 100 BP support for both subgenera), whereas eight increased by 1–8 BP and four decreased by 8–13 BP relative to support values for the same clades in the parsimony analysis of molecular data alone.

DISCUSSION

IMPLICATIONS OF ANALYSES OF MORPHOLOGICAL DATA ON SUBGENERIC TAXONOMY AND PHYLOGENY OF *OURISIA*

As expected, none of our analyses of the morphological data supported the delimitation of subgenera based on the characters used by Poeppig and Endlicher (1835), namely calyx symmetry (character 7), corolla curvature, and corolla lobe orientation (character 14). These latter two characters are not correlated with calyx symmetry in any species (except *Ourisia ruellioides*), and all three characters are homoplastic when mapped onto the phylogeny (data not shown). In contrast, delimitation of two subgenera within *Ourisia* based on habit (character 1; Meudt, 2006) is highly supported by phylogenetic analysis of morphological and combined data (e.g., Fig. 2). At least six other morphological characters, including leaf attachment (character 2), inflorescence type (character 5), calyx internal glandular vestiture (character 8), ovary vestiture (character 17), hypogynous disc (character 18), and seed type (character 19), support this infrageneric taxonomy. All of these characters (except seed type) have character states that are synapomorphies for the 25 species in *Ourisia* subg. *Ourisia*, i.e., herbaceous habit, petiolate leaves, bracteate inflorescences, glabrous or sessile glandular trichomes inside calyx and on the ovary, and a reduced hypogynous disc (characters 1, 2, 5, and 18 shown in Fig. 3).

Within *Ourisia* subg. *Ourisia*, the New Zealand herbaceous species are monophyletic in both the molecular and combined analyses (Figs. 1 and 2), and are linked by two synapomorphies: $2n = 48$ chromosomes (character 20) and New Zealand–type seed coat (character 19; with the exception of *O. modesta* Diels with autapomorphic *modesta*-type seeds). Support for most relationships within the New Zealand clade remain the same or are slightly higher when morphological and molecular data are analyzed together. Analysis of the combined data set shows that the Andean *O. alpina* Poepp. & Endl. and *O. coccinea* are sister to the Australasian species (Fig. 2), which contrasts with the Bayesian analysis of the molecular data that supports a monophyletic herbaceous Andean clade that is sister to the Australasian species (Fig. 1). There are no morphological synapomorphies for a herbaceous Andean clade. Finally, the addition of morphological data to the analysis does not support the monophyly of the four sampled north-central Andean species (Fig. 2). Three of these species—*O. cotapatensis* Meudt & S. Beck, *O. pulchella* Wedd., and *O. chamaedrifolia* Benth.—are strongly supported as monophyletic in both molecular (97 BP, 1.0 PP) and combined (88 BP)

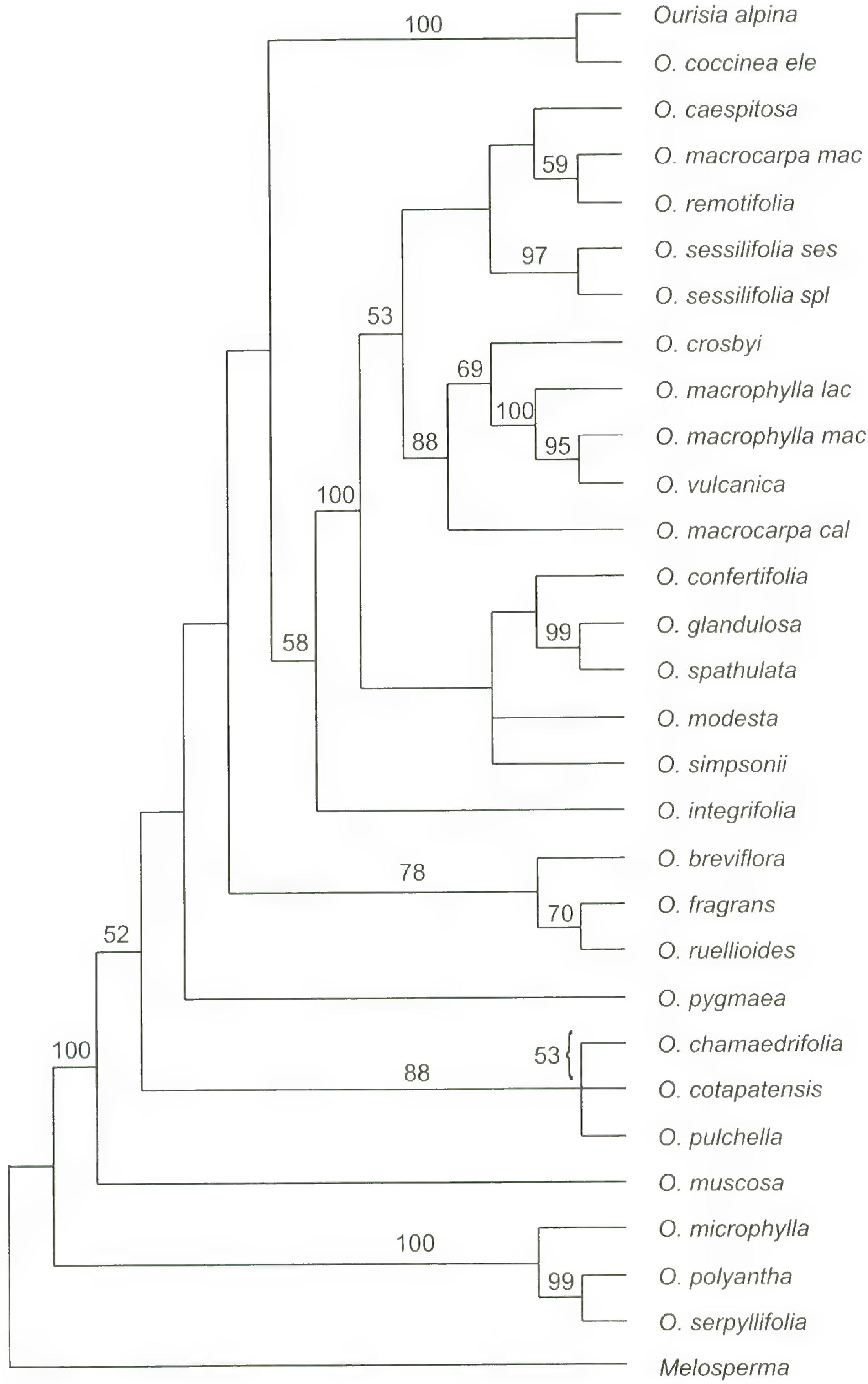

Figure 2. Strict consensus tree of the nine MPTs from parsimony analysis of the *Ourisia* combined (molecular plus morphological) data of 1372 steps. BP values > 50 are shown above the branches. The bracket { indicates 53 BP for the sister relationship of *O. chamaedrifolia* and *O. cotapatensis*, but these two species form part of a polytomy in the strict consensus tree shown here. Refer to Figure 1 for subspecies names.

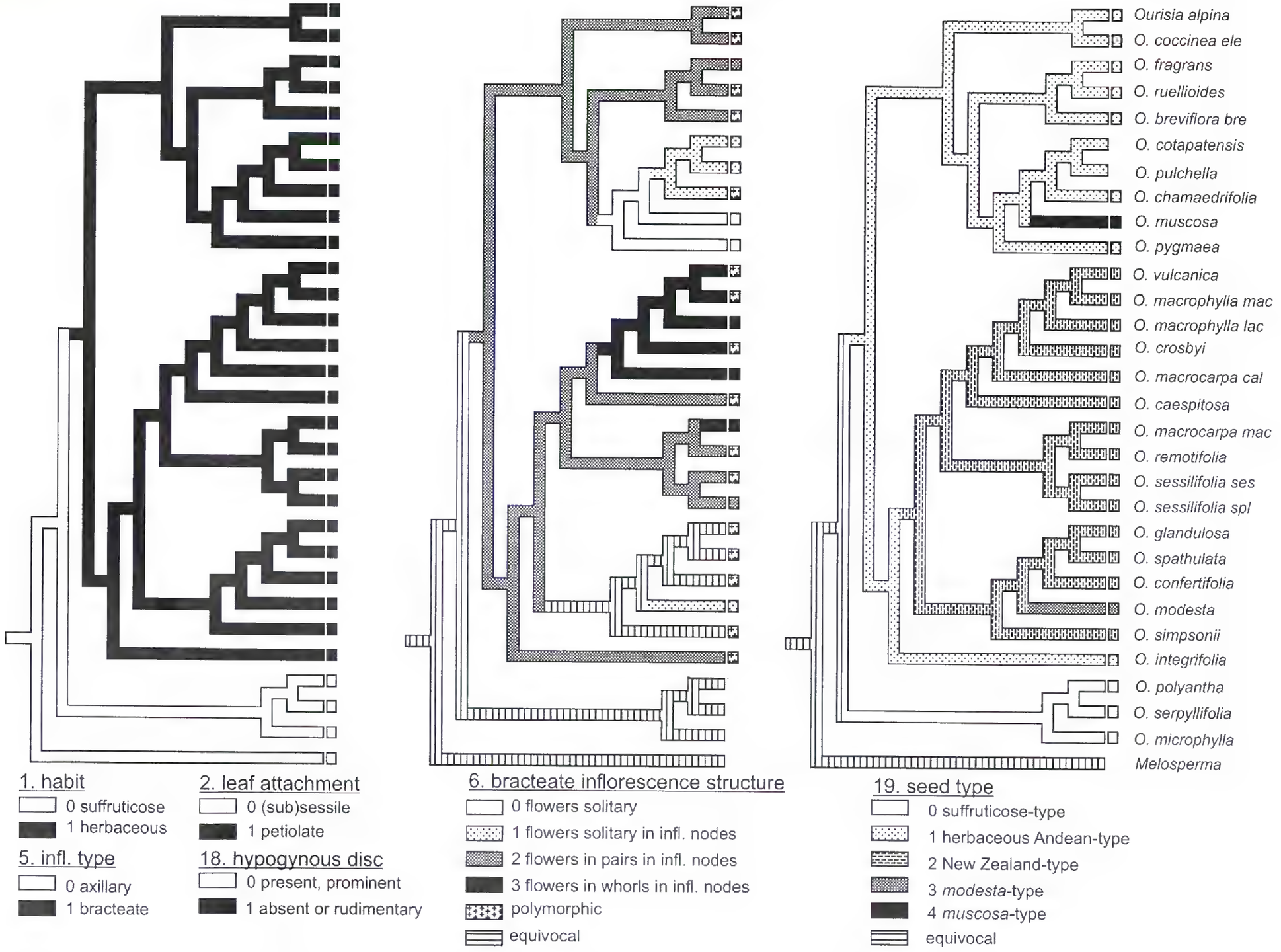

Figure 3. Morphological character evolution of selected characters in *Ourisia*. Characters 1, 2, 5, 18, and 19 support delimitation of two subgenera, *Ourisia* and *Suffruticosae*; character 6 is also shown. All characters are mapped onto the Bayesian tree of the molecular data set (Fig. 1). For a detailed explanation of characters and character states, see Appendix 1; for the character matrix, see Table 2. Refer to Figure 1 for subspecies names.

analyses. Whereas *O. muscosa* is strongly supported as sister to these three species in the molecular-only analysis (1.0 PP, Fig. 1), it does not form part of this clade in the combined analysis (Fig. 2). The north-central Andean species do not share any morphological synapomorphies, in part due to the unique morphology of *O. muscosa*, which is a minute species with solitary white, tubular, regular flowers and a dense vestiture of yellow trichomes inside the corolla tube that sets it apart from the other four species. *Ourisia muscosa* has two characters that are unique in the genus: calyces covered by a pair of subtending bracts and autapomorphic *muscosa*-type seeds. As *O. muscosa* reaches the highest elevation (5000 m) of any species of *Ourisia*, a plausible hypothesis for its unique characters is that there has been strong selection for morphological features adapted to these extreme environments.

Turning now to *Ourisia* subg. *Suffruticosae*, there are at least two possible synapomorphies for this clade (ovary with stalked glandular vestiture and suffruticose-type seeds, Fig. 3); however, the states for these characters in the outgroup are unknown. *Ourisia* subg. *Suffruticosae* shares at least five pleisiomorphic character states with the outgroup, *Melosperma*, namely woody habit, sessile leaves, solitary, axillary flowers, presence of a prominent hypogynous disc (characters 1, 2, 5, and 18 in Fig. 3), and stalked glandular hairs on the calyx (internally). These could represent the ancestral character states for *Ourisia*, but analyses with additional outgroups are needed.

Within *Ourisia* subg. *Suffruticosae*, analyses of morphological data show that relationships in this clade are either completely unresolved (morphology alone, not shown) or identical to those resolved in the molecular data tree (combined, Fig. 2). The three species have striking floral morphological differences but are very similar vegetatively. In fact *O. microphylla* and *O. polyantha* are only readily distinguished based on floral characters. However, *O. serpyllifolia* (not *O. polyantha*) was found to be sister to *O. microphylla* (Figs. 1, 2). Numerous autapomorphic characters distinguish *O. serpyllifolia* from all other species of *Ourisia*, including anthers with divaricate, explanate thecae, a style that is distinctively bent near the stigma, and stamens that are uniquely curved toward one another. Although all three suffruticose species occur in similar habitats in the southern Andes, *O. serpyllifolia* (29°–33°S, plus one disjunct locality at 36°S) is almost entirely allopatric to *O. polyantha* (33°–35°S) and *O. microphylla* (35°–38°S) (Meudt, 2006). It would be interesting to investigate whether differing pollinator faunas in these areas have resulted in specialization to different pollinators that are driving morphological divergence in these species, particularly in *O. serpyllifolia*.

PATTERNS OF EVOLUTION OF SELECTED MORPHOLOGICAL CHARACTERS OF INTEREST

Inflorescence type. Bracteate inflorescences in *Ourisia* subg. *Ourisia* have evolved from solitary, axillary flowers subtended by leaves found in *Ourisia* subg. *Suffruticosae* and the outgroup, *Melosperma* (character 5, Fig. 3). Within *Ourisia* subg. *Ourisia*, bracteate inflorescences with pairs of flowers in the inflorescence nodes appear to be ancestral (character 6, Fig. 3). Inflorescences with a reduced number of flowers and those with whorls of flowers have both evolved on separate occasions in the Andes and Australasia. In New Zealand, the seven taxa that can have inflorescences with whorled flowers in the inflorescence nodes belong to two different clades.

Seed morphology. *Ourisia* seeds are very small (≤ 2.0 × ≤ 1.1 mm) and have a glabrous, usually bireticulate seed coat that is superficially similar to seed coats in *Gratiola*, *Mecardonia*, *Melosperma*, *Bacopa*, and other genera of Gratioleae and other tribes (Meudt, 2006). Differences in seed coat morphology (character 19) can be used to distinguish subgenera in *Ourisia* (see Meudt, 2006 and Webb & Simpson, 2001 for SEM photographs of seeds). Suffruticose-type seeds are very distinctive from all other seed types and are only found in species of *Ourisia* subg. *Suffruticosae*. In contrast, there are four different seed types represented in species of *Ourisia* subg. *Ourisia*: herbaceous Andean–type, New Zealand–type, *muscosa*-type, and *modesta*-type. In our analysis, New Zealand–type, *modesta*-type, and *muscosa*-type seeds are all derived from the herbaceous Andean–type seeds (character 19, Fig. 3). A more detailed survey of seed coat morphological characters for related genera is needed to determine the ancestral seed type within *Ourisia*, which is equivocal.

Chromosome number. The base chromosome number (character 20) in *Ourisia* is $x = 8$, and all 12 New Zealand species have been determined to be hexaploids at $2n = 48$. The sole Tasmanian species, *O. integrifolia*, is $2n = 32$ (Hair & Arroyo, 1984), whereas the few Andean species that have counts (three out of 15) are $2n = 16$ (see Meudt, 2006), including *O. microphylla*, which also has been counted at $2n = 32$ (Rossow, 1993). When mapped onto the molecular phylogeny (not shown), chromosome number and ploidy levels show, as expected, an increasing trend from diploidy ($2n = 16$) to hexaploidy ($2n = 48$). This pattern of chromosome numbers may support the idea that a polyploidization

event accompanied the colonization of Australasia by diploid South American ancestors of *Ourisia* (Meudt & Simpson, 2006). It is not known whether this event was due to allopolyploidy or autopolyploidy. If polyploidization occurred after long-distance dispersal of *Ourisia* to Australasia, perhaps autopolyploidy is more the likely scenario, as it only requires the dispersal of one individual. On the other hand, some species of *Ourisia* are interfertile and can produce natural (Meudt, 2006) or artificial (e.g., Sheader & Sheader, 2004) hybrids. More chromosome studies in *Ourisia* are needed to determine the extent to which polyploidy has occurred in the Andes and which Andean species have karyotypes most similar to the Australasian species.

Corolla characters. Several floral morphological characters are of interest with respect to character evolution within *Ourisia* (Fig. 4). Regarding internal corolla tube vestiture, trichomes have evolved four times from the ancestral glabrous state (character 12, Fig. 4). These transitions have occurred in both the north-central Andes and New Zealand. A monophyletic group of seven New Zealand species has three lines of trichomes inside the corolla tube, with the exception of *O. sessilifolia* Hook. f. subsp. *splendida* (L. B. Moore) Arroyo, which only has one line. The one line of hairs inside the corolla tube of *O. modesta* has apparently evolved independently within the clade that comprises the remaining New Zealand species, all with glabrous corolla tubes.

Red corollas have evolved on four separate occasions in Andean *Ourisia*, e.g., *O. polyantha*, *O. ruellioides*, *O. chamaedrifolia*, and *O. coccinea*, from white or light pink/light purple corollas (character 9, Fig. 4). These four species, which are not closely related to one another, have flowers that are also typically very long, with longer styles and stamens than those of other species of *Ourisia*. Long, red, tubular or tubular-funnelform corollas are often cited as indications of a hummingbird-pollinated syndrome (Grant & Grant, 1968). *Ourisia ruellioides* was studied with respect to its pollination biology and found to be self-compatible and strongly autogamous, with flowers that produce abundant nectar (Arroyo & Peñaloza, 1990). No pollinators were seen visiting this species during the course of their study (Arroyo & Peñaloza, 1990), which was conducted in the extreme south of Chile where pollinators may be less active and/or less abundant than at localities farther north (Arroyo et al., 1985).

The remaining species of *Ourisia* have light pink to light purple or white corollas with comparatively short corolla tubes. Many of these species have contrasting colors such as yellow or dark purple inside the corolla tube (character 11), often in the form of lines or spots, as well as some type of vestiture (characters 12 and 13) that may function as nectar guides for insect pollinators. Yellow inside the corolla tube is the most common state for this character and appears to be ancestral in *Ourisia* (character 11, Fig. 4). This contrasting color has been lost and regained in the Andes, but largely retained in most New Zealand species. Purple inside the corolla tube has also evolved once in the Andes (*O. cotapatensis*) and at least three times in New Zealand, i.e., *O. sessilifolia* and *O. remotifolia* Arroyo (purple only), *O. glandulosa* Hook. f. (purple and yellow), and *O. simpsonii* (L. B. Moore) Arroyo (purple and yellow) (character 11, Fig. 4). All species in New Zealand have small, white, weakly zygomorphic flowers that appear to be adapted to the unspecialized pollinator fauna there (Lloyd, 1985). For example, small generalist bees and flies have been observed pollinating the flowers of *O. macrocarpa* Hook. f. subsp. *calycina* (Colenso) Arroyo from New Zealand, which also contained trace amounts of nectar (Schlessman, 1986). Given the generalist alpine pollinator fauna of New Zealand, floral differences among the New Zealand species are particularly interesting and may suggest that pollinators have been a more important selective force than previously hypothesized in the evolutionary history of *Ourisia*.

Whether species of *Ourisia* with similar floral characteristics have similar pollinators, and whether these pollinators have driven the evolution of floral morphology in *Ourisia* are interesting questions that must be addressed by combining investigations of pollination biology and phenotypic selection with phylogenetics, as has been done recently in *Schizanthus* Ruiz & Pav. (Pérez et al., 2006) and *Penstemon* Schmidel (Wilson et al., 2006). It was shown in both of these studies that bird pollination has recently evolved numerous times (and at high elevation in *Schizanthus*) from bee-pollinated ancestors. These studies are particularly relevant here because *Penstemon* is a member of the same family as *Ourisia* and *Schizanthus* has a very similar distribution to *Ourisia* in the South American Andes. The finding that white, light pink, or light purple corollas appear to be ancestral and that red corollas appear to be derived in *Ourisia* (character 9, Fig. 4) also agrees with the hypothesis that, in general, insect-pollinated (and especially bee-pollinated), bell-shaped flowers are ancestral in Scrophulariaceae s.l. (Kampny, 1995). Although Scrophulariaceae s.l. is now known to be polyphyletic, this hypothesis can still be considered valid in the case of *Ourisia* because four of the tribes discussed by Kampny (1995) as having the ancestral bell-shaped flowers now form the core of a recircumscribed Plantaginaceae (Albach et al., 2005). There is also some evidence that bee pollination may even be ancestral in other closely related families in Lamiales (Kampny, 1995).

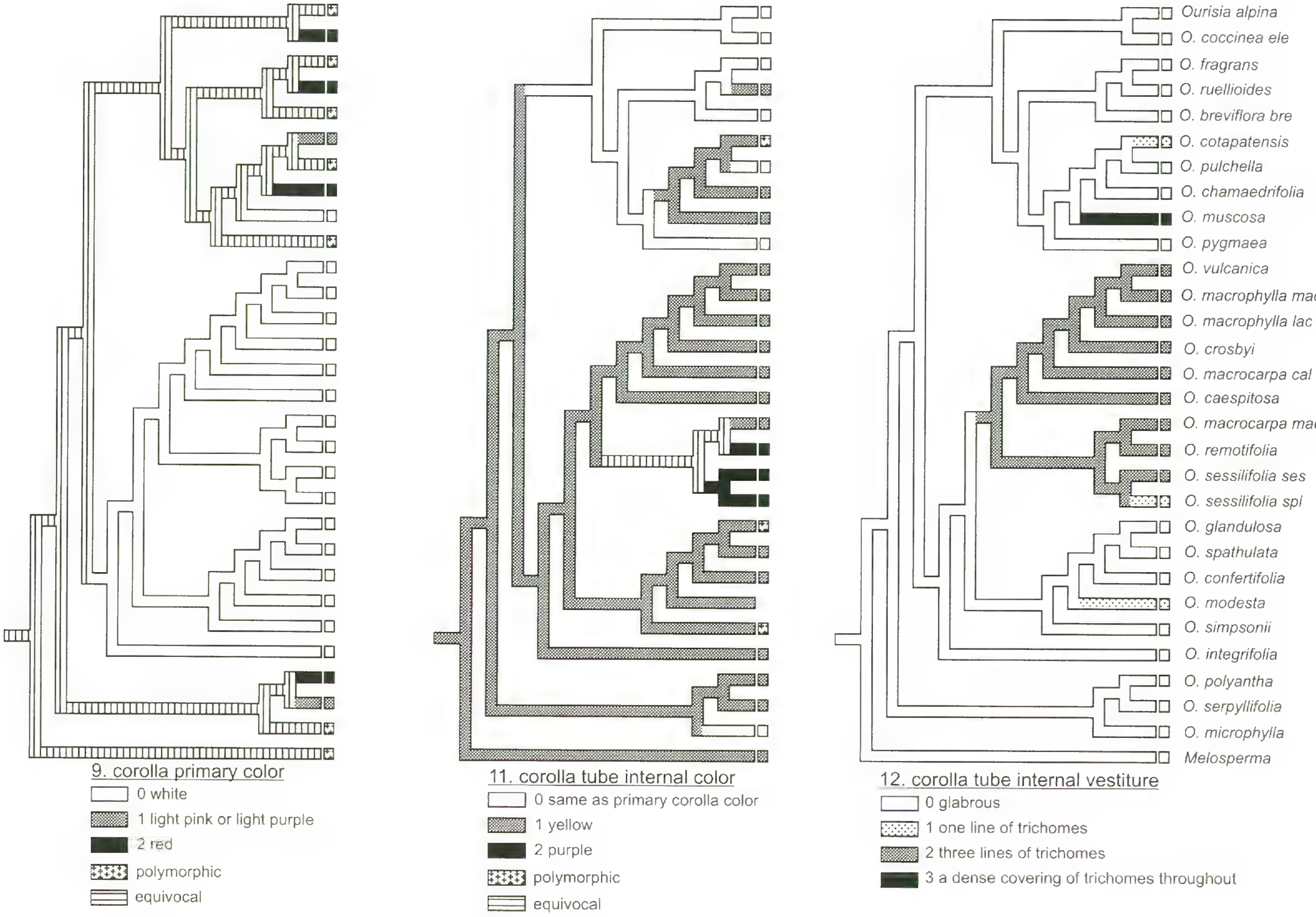

Figure 4. Morphological character evolution of selected characters in *Ourisia*. Characters 9, 11, and 12 represent selected floral characters discussed in the text. All characters are mapped onto the Bayesian topology of the molecular data set (Fig. 1). For an explanation of characters and character states, see Appendix 1; for the character matrix, see Table 2. Refer to Figure 1 for subspecies names.

In addition to being an ideal candidate for studying the evolution of floral features, breeding systems, and pollinators, *Ourisia* could be particularly informative regarding comparative studies between mountainous areas with differing pollinator faunas such as those in New Zealand and South America, or in the southern Andes alone, where at least three highly supported clades (Fig. 1) contain species with widely differing floral morphologies (e.g., *O. coccinea* and *O. alpina*; *O. breviflora*, *O. fragrans* Phil., and *O. ruellioides*; and *O. microphylla*, *O. polyantha*, and *O. serpyllifolia*).

Summary

In summary, phylogenetic analyses of molecular and morphological characters both show high support for two monophyletic subgenera within *Ourisia*, with a notable number of morphological synapomorphies for the two subgenera. Morphological data supported or provided synapomorphies for some relationships within *Ourisia* subg. *Ourisia*, such as the New Zealand clade, but not for others, such as the herbaceous Andean species. Within the genus as a whole, and using *Melosperma* as an outgroup, analyses of morphological data elucidated several trends, including evolution from light-colored to red-colored corollas and glabrous to hairy corollas (both numerous times), simple to more complex inflorescences, and lower to higher ploidy levels. Seed coat morphology is particularly useful for defining groups within *Ourisia* and may be an important character for studies within Plantaginaceae as a whole, although future molecular and morphological phylogenetic studies for all plantaginaceous genera are necessary and warranted. *Ourisia* is an ideal candidate for further integrated studies regarding the evolution of floral features, breeding systems, and pollinators and could provide unique and comparative insights between species in mountainous areas of South America and Australasia.

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Appendix 1. Characters and states of the *Ourisia* morphological data set.

1. habit: 0, suffruticose with many branches arising from woody base; 1, herbaceous with repent stems.

2. leaf attachment: 0, sessile or subsessile; 1, petiolate.

3. petiole type: 0, petiole distinct from lamina; 1, petiole broad and difficult to distinguish where it begins and lamina ends. The common state among petiolate species of *Ourisia* is 0. This character was inapplicable for species with sessile leaves (character 2, state 0) and were thus coded as missing data in PAUP*.

4. leaf margin: 0, entire to subentire, or irregularly notched; 1, regularly crenate; 2, regularly serrate. A few taxa are variable, i.e., 01, with subentire to regularly crenate leaves.

5. inflorescence type: 0, solitary, axillary flowers subtended by leaves; 1, bracteate inflorescence.

6. inflorescence structure of bracteate inflorescences: 0, flowers solitary (never more than one flower per inflorescence); 1, flowers solitary in inflorescence nodes; 2, flowers in pairs in inflorescence nodes; 3, flowers in whorls in inflorescence nodes. There is variation within many taxa for inflorescence structure, resulting in taxa being coded polymorphic, either as 12 or 23. For example, *Ourisia alpina* usually has paired flowers in each node (state 2), but occasionally plants with whorled flowers in each node (state 3) can be found. This character was inapplicable for taxa that do not have bracteate inflorescences (character 5, state 0) and were thus coded as missing data in PAUP*.

7. calyx symmetry: 0, irregular, the three posterior lobes and two anterior calyx lobes divided to differing lengths; 1, regular or subregular, all calyx lobes divided all the way to base; 2, regular or subregular, all calyx lobes divided to ca. 1/2 the length of the calyx. Some taxa were variable for this character, i.e., 01 or 02. Regular calyces could have either all calyx lobes divided all the way to the base (i.e., a very short calyx tube, state 1) or all calyx lobes divided to ca. 1/2 the length of the calyx (i.e., a long calyx tube, state 2).

8. calyx internal glandular vestiture: 0, glabrous; 1, with sessile glandular trichomes; 2, with stalked glandular trichomes. Glandular trichomes may be sessile or stalked, but not both. All taxa with sessile glandular trichomes (state 1) were always variable, i.e., 01, and some taxa with stalked glandular trichomes were variable, i.e., 02.

9. corolla primary color: 0, white; 1, light pink or light purple; 2, red. Many Andean taxa are variable for white and light pink or purple corollas, i.e., 01, while other taxa have only white or only light pink/light purple corollas.

10. corolla symmetry: 0, zygomorphic; 1, regular or subregular. Corollas in the family are largely zygomorphic. The corolla symmetry of *Ourisia integrifolia* can be subregular to weakly zygomorphic and was thus coded as polymorphic, i.e., 01.

11. corolla tube internal color: 0, same as primary corolla color; 1, yellow; 2, purple. Some taxa with both yellow and purple inside the corolla tube were coded as polymorphic, i.e., 12.

12. corolla tube internal vestiture: 0, glabrous; 1, one line of trichomes; 2, three lines of trichomes; 3, a dense covering of trichomes throughout.

13. corolla tube vestiture at tube opening: 0, absent (glabrous); 1, present (trichomes more or less forming a ring). Some taxa are variable for this character, i.e., 01.

14. corolla lobe orientation: 0, widely spreading; 1, not spreading or only weakly spreading.

15. filament base vestiture: 0, glabrous; 1, patch of sessile glandular trichomes on two long stamens; 2, patch of sessile glandular trichomes on all four stamens; 3, sessile glandular trichomes in a ring around corolla tube base. Many taxa are variable for this character, with two states or, as in the case of *Ourisia microphylla*, even all four states present.

16. staminode: 0, absent; 1, present and rudimentary; 2, present as a fifth, fertile stamen. All taxa with a rudimentary staminode were always variable. The variable condition 01 was the most common. *Ourisia fragrans* always has five fertile stamens (state 2), while *O. chamaedrifolia* and *O. caespitosa* were coded as polymorphic for this character because, although they almost always have rudimentary staminodes, one to a few specimens were found with a fifth stamen, i.e., 12.

17. ovary vestiture: 0, glabrous; 1, with sessile glandular trichomes; 2, with stalked glandular trichomes. This vestiture character is similar to character 8, calyx internal glandular vestiture. Glandular trichomes may be sessile or stalked, but not both. All taxa with sessile glandular trichomes were always variable, i.e., 01.

18. hypogynous disc: 0, present, obvious, prominent; 1, absent or rudimentary, not prominent. The hypogynous disc is a ring of tissue around the base of the ovary that remains attached to the ovary when the corolla is shed. In some species, the disc is not prominent and can be difficult to assess in herbarium specimens. It is likely a nectary, but its anatomy needs to be investigated in more detail. Nectar has been found in the flowers of two herbaceous species of *Ourisia* that have been studied with respect to reproductive and pollination biology, including trace amounts in *O. macrocarpa* (Schlessman, 1986) and abundant nectar in *O. ruellioides* (Arroyo & Peñaloza, 1990). *Melosperma* was coded as 0 for this character because it contains a very similar hypogynous disc nectary (together with its sister genus *Monttea*; Sérsic & Cocucci, 1999).

19. seed type: 0, suffruticose-type; 1, herbaceous Andean type; 2, New Zealand–type; 3, *modesta*-type; 4, *muscosa*-type.

20. *2n* chromosome number: 0, 16; 1, 32; 2, 48. Most of the South American species have chromosome numbers that are unknown for this character.

REVISIÓN TAXONÓMICA DE LAS ESPECIES DEL GÉNERO *VERBENA* (VERBENACEAE): SERIE *PACHYSTACHYAE*[1]

Nataly O'Leary,[2] *María Ema Múlgura,*[2] *and Osvaldo Morrone*[2]

Resumen

Verbena L. comprende ca. 45 especies; el género es mayoritariamente americano con unas pocas especies de distribución mundial. Se presenta un tratamiento taxonómico para el género *Verbena* serie *Pachystachyae* Schauer; esta serie incluye 18 especies de distribución principalmente sudamericana. En el presente tratamiento se trata bajo la serie *Pachystachyae* dos subseries: *Pachystachyae* y la nueva subserie *Pseudoracemosae* N. O'Leary, estableciendo los caracteres diagnósticos de las mismas. Se proporcionan descripciones detalladas de todos los taxones, claves para identificarlos, ilustraciones, una sinonimia actualizada con 43 nuevos sinónimos, y se discuten las relaciones entre taxones afines. Se introducen dos nuevas combinaciones: *Verbena hispida* var. *obovata* (Moldenke) N. O'Leary y *V. litoralis* var. *brevibracteata* (Kuntze) N. O'Leary; un nombre nuevo: *V. subpetiolata* N. O'Leary; se funda la subserie nueva *Pseudoracemosae*; y se presenta la lectotipificación de *V. bonariensis* L. f. *robustior* Chodat, *V. buchnera* Vell. y *V. lobata* Vell.

Abstract

Verbena L. is represented by ca. 45 species; the genus is mainly American with a few species of worldwide distribution. A taxonomic treatment of the genus *Verbena* series *Pachystachyae* Schauer is here proposed; this series includes 18 species, the majority of South American distribution. In the present treatment, under the series *Pachystachyae*, two subseries are treated and their diagnostic characters are given: *Pachystachyae* and the new subseries *Pseudoracemosae* N. O'Leary. Detailed morphological descriptions are given for each taxon, as well as keys for their identification, illustrations, up-to-date synonymy with 43 new synonymized species, and discussion of relationships between closely related taxa. Two new combinations are introduced: *Verbena hispida* var. *obovata* (Moldenke) N. O'Leary and *V. litoralis* var. *brevibracteata* (Kuntze) N. O'Leary; the new name *V. subpetiolata* N. O'Leary is established; the new subseries *Pseudoracemosae* is established; and the species *V. bonariensis* L. f. *robustior* Chodat, *V. buchnera* Vell., and *V. lobata* Vell. are lectotypified.

Key words: Nomenclature, *Pachystachyae*, South America, taxonomy, *Verbena*, Verbenaceae.

Verbena L. (Verbenaceae) fue fundado por Linnaeus (1753) abarcando 14 especies, de las cuales 6 actualmente pertenecen a *Verbena* (*V. bonariensis*, *V. hastata*, *V. officinalis*, *V. spuria*, *V. supina* y *V. urticaefolia*); las restantes fueron transferidas a otros géneros. Comprende hierbas o sufrútices, algunas de ellas importantes como ornamentales y otras como malezas. *Verbena* se distribuye principalmente en América, de donde el género sería originario (Lewis & Oliver, 1961). Varias de las especies poseen distribución cosmopolita, como *V. rigida* Spreng., *V. litoralis* Kunth y *V. bonariensis* L.

Moldenke (1983) menciona ca. 450 especies y taxones infraespecíficos para *Verbena*; autores más recientes sugieren que habría 300 especies de *Verbena* (Méndez Santos, 2003), entre 200 y 250 especies (Atkins, 2004) y ca. 100 especies (Rzedowski & Rzedowski, 2002). Nuestros estudios hasta el momento indican la presencia de ca. 45 especies, coincidiendo con Sanders (2001) que estima entre 40 y 50 especies. En su monografía sobre el género *Verbena*, Moldenke (1947–1982) prácticamente duplicó el número de nombres que existían bajo *Verbena*, describiendo nuevas especies que no se hallan claramente delimitadas ya que carece de claves para su identificación. Hasta el momento, los trabajos en *Verbena* distan de clarificar las complejas relaciones dentro del género y únicamente se cuenta con estudios a nivel regional o catálogos, como Perry (1933) para Norteamérica; Gibson (1970) para Guatemala; López-Palacios (1977) para Venezuela; Brako y Zarucchi (1993) para Perú; Múlgura (1999) para Argentina;

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Jørgensen y León-Yánez (1999) para Ecuador; Munir (2002) para Australia y Rzedowski y Rzedowski (2002) para México.

En América del Sur, donde el género presenta una importante diversidad de especies, diferentes de las que se encuentran en América del Norte (Sanders, 2001), los estudios taxonómicos llevados a cabo hasta el presente son parciales, no se cuenta con una revisión en conjunto del mismo y no se han planteado ni resuelto los problemas de relación entre especies y grupos infragenéricos.

El objetivo del presente trabajo es realizar la revisión taxonómica de las especies de la serie *Pachystachyae* Schauer del género *Verbena*. Se propone establecer caracteres diagnósticos a nivel de los grupos infragenéricos y de sus especies sobre la base de datos exomorfológicos y anatómicos. El trabajo aporta descripciones, ilustraciones, claves para la identificación de las especies y preferencias ecológicas de las mismas.

Historia Taxonómica y Delimitación del Género

La historia de *Verbena* ha estado muy ligada a la del género afin *Glandularia*, fundado por Gmelin en 1791. Originalmente Schauer (1847) estableció la sección *Glandularia* dentro del género *Verbena* caracterizada por la presencia de apéndices glandulares en las anteras de los dos estambres superiores. Numerosos autores siguieron tratando *Glandularia* como serie, sección, o subgénero de *Verbena* (Briquet, 1895; Perry, 1933; Moldenke, 1940, 1942a, 1942b, 1971, 1973, 1983, entre otras; Macbride, 1960; Lewis & Oliver, 1961; Raj, 1983; Munir, 2002). Otros autores aceptaron *Glandularia* como género independiente de *Verbena* (Small, 1933; Schnack & Covas, 1944; Troncoso, 1974; Umber, 1979; Sanders, 2001) sobre la base de la morfología de las florescencias y caracteres citogenéticos, fitoquímicos y anatómicos. Consecuentemente muchas de las especies previamente tratadas bajo *Verbena* han sido transferidas a *Glandularia* (Schnack & Covas, 1944; Troncoso, 1974; Umber, 1979).

Moldenke en 1940 funda el género *Junellia* caracterizado por ser plantas de hábito leñoso con ramas retorcidas, las hojas imbricadas y con frecuencia rígidas y espinosas, o reducidas a escamas, las florescencias paucifloras, muchas veces solitarias. Troncoso (1974) trata a *Junellia* como una sección de *Verbena*; posteriormente Botta (1989: 382) acepta *Junellia* como género separado de *Verbena* sobre la base del hábito leñoso y la morfología foliar. Consecuentemente, muchas especies de *Verbena* han sido transferidas a *Junellia* por los autores que reconocen estos dos géneros como diferentes (Moldenke, 1940; Botta, 1989).

Lewis y Oliver (1961), al estudiar la frecuencia de los niveles de ploidía en especies de *Verbena* y de *Glandularia*, propusieron que *Verbena* se habría originado en Norteamérica y habría migrado a Sudamérica donde ya estaría establecido *Glandularia*, que habría surgido en Sudamérica. Por lo tanto, según Lewis y Oliver (1961), la poliploidía habría sido el mecanismo de especiación de las verbenas de Sudamérica, que habrían tenido que competir con las glandularias nativas. Por otro lado, estudios cromosómicos en *Junellia* (Botta & Brandham, 1993) confirmarían que *Junellia* y *Glandularia* estarían más cercanamente relacionados entre sí que con *Verbena*, lo cual se condice con estudios morfológicos y de distribución para ambos géneros (Botta, 1989).

Subdivisión del Género

Sprengel (1825) fue el primer autor en dividir el género *Verbena* en dos grupos informales: con hojas indivisas y con hojas trífidas. Hooker (1829) también dividió las especies en dos grupos informales pero sobre la base de la corola 4-fida ó 5-fida. Schauer (1847) lo dividió en dos secciones: sect. *Glandularia* (J. F. Gmel.) Schauer y sect. *Verbenaca* Schauer; esta última estaría integrada por 55 especies agrupadas en 5 series: las series *Pungentes* Schauer, *Nobiles* Schauer y *Verticilliflorae* Schauer, actualmente tratadas bajo *Junellia* y *Glandularia* (Troncoso, 1974; Botta, 1989), y las series *Pachystachyae* Schauer y *Leptostachyae* Schauer. Schauer (1847), al fundar la serie *Pachystachyae*, la caracterizó por poseer flores en "cabezuelas" comprimidas o "espigas" y hojas grandes, mientras que la serie *Leptostachyae* poseería "espigas" filiformes, elongadas, gráciles. Posteriormente Briquet, en 1895, siguiendo el criterio de Schauer, agregó dos series más dentro de la sect. *Verbenaca*, *Junciformis* Briq. y *Acerosae* Briq., que actualmente se incluyen en el género *Junellia* (Botta, 1989). Perry (1933) en su tratamiento taxonómico de *Verbena* para Norteamérica caracterizó la serie *Pachystachyae* por poseer florescencias breves y congestas en antesis, nunca muy elongadas o abiertas, generalmente dispuestas en "cimas complejas" (Perry, 1933: 252). Este autor reconoció también la serie *Leptostachyae* y la caracterizó por sus florescencias delgadas, abiertas o compactas en antesis, elongándose mucho en fructificación, solitarias o dispuestas en "cimas complejas" (Perry, 1933: 259). Troncoso (1974) definió las especies de la serie *Pachystachyae* por poseer florescencias cilíndricas cuando jóvenes, densas y breves, con flores aproximadas, luego alargándose en antesis, distinguiéndo la serie *Leptostachyae* por sus florescencias filiformes, gráciles y flores distanciadas, muy alargadas después de la

antesis. Sanders (2001), para definir la serie *Pachystachyae*, habló de florescencias breves, compactas, cilíndricas; flores y frutos imbricados densamente de manera espiralada; florescencias agregadas en "dicasios paniculosos de tres a cinco órdenes de ramaje" (Sanders, 2001: 335). La serie *Verbena* [= *Leptostachyae* Schauer] se caracterizaría por florescencias que se elongan por crecimiento apical, flores y frutos contiguos o separados remotos, subopuestos o espiralados de manera espaciada; florescencias agregadas en "dicasios paniculosos de uno o dos órdenes de ramaje, o solitarias" (Sanders, 2001: 335).

Materiales y Métodos

Se estudiaron aproximadamente 1600 ejemplares, depositados en los herbarios AS, BA, BAB, BAF, CORD, CTES, FCQ, LP, MO, NY, P, PY, SI y US (abreviaturas según Holmgren et al., 1990). Las listas de las especies y variedades aceptadas y de los ejemplares examinados se proveen en los Apéndices 1 y 2. En Apéndice 3 se provee la lista de las especies sinonimizadas en este trabajo con indicación de su actual ubicación taxonómica.

Nomenclatura. La identificación de las especies se hizo por comparación con el material tipo o fotografías del mismo, lo mismo que la resolución de los sinónimos taxonómicos. La cita de los tipos se refiere exactamente a lo que se lee en la/s etiqueta/s de herbario que posea el ejemplar, a excepción que se aclare lo contrario. El Instituto de Botánica Darwinion cuenta con las fotografías de ejemplares tipo de Verbenaceae de la colección de MacBride del Field Museum of Natural History de Chicago; esto se indica con la abreviatura de herbario donde está o estaba depositado el tipo seguida del número de la foto de la serie de MacBride, ej.: "B foto F número".

Las flores se describieron en antesis, las dimensiones del cáliz se presentan como longitud para referirse a la medida desde el extremo de inserción al raquis hasta el margen distal. Las mediciones de longitud de las clusas se tomaron, siempre que fue posible, a partir de frutos maduros. Para la descripción del hábito se usó la terminología de Cabrera (1957). Se siguió a Lindley (1951), empleando su criterio para la definición del grado de división de las láminas foliares; para la morfología general de las hojas se siguió el criterio de Hickey (1974: 9). Para la descripción de la pubescencia se siguió a Lawrence (1951: figs. 308, 309).

En los estudios anatómicos se utilizó material de herbario, el cual fue hervido unos minutos en agua con unas gotas de detergente, dejándolo sumergido en esta solución 48 horas y transfiriéndolo después a FAA. Los cortes fueron realizados a mano alzada, se aclararon con solución diluída de hipoclorito de sodio y se tiñeron con safranina en solución acuosa (D'Ambroggio, 1986).

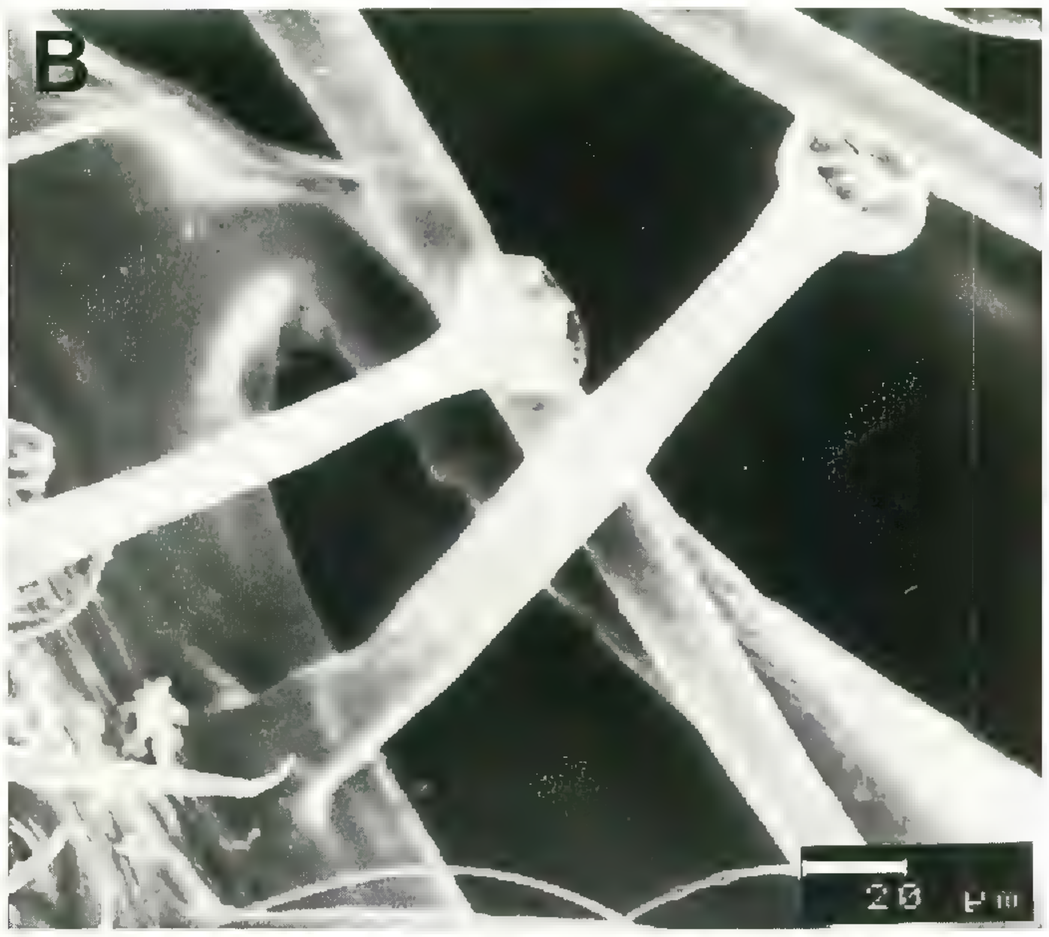
Las fotomicrografías de pelos y pubescencia se realizaron de acuerdo a los procedimientos descriptos por Zanotti y Pozner (2005). Se empleó un microscopio electrónico de barrido (MEB) Zeiss 940A, perteneciente al Instituto de Botánica Darwinion; y se operó a 10–20 kV.

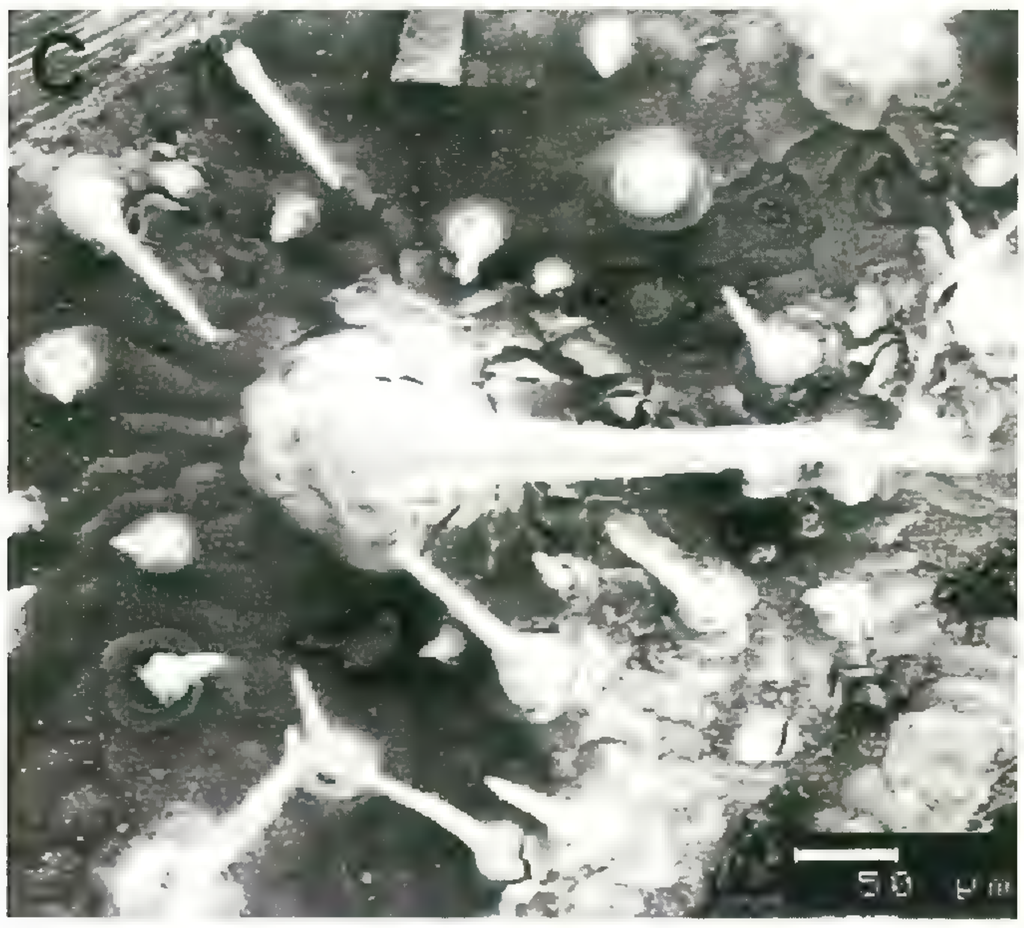
Morfología

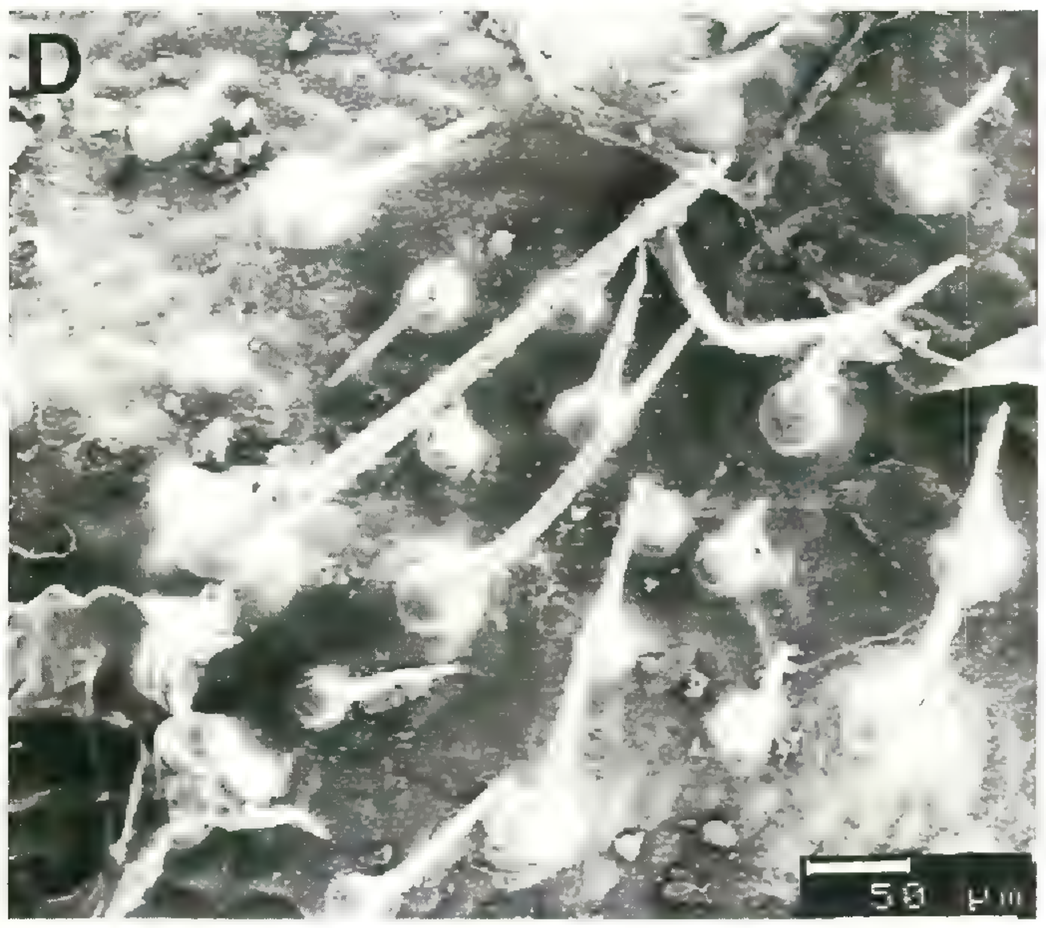
Hábito. Las especies son hierbas o sufrútices, erectas o decumbentes, a veces rastreras, con yemas de renuevo cerca del nivel del suelo. En general los órganos aéreos mueren durante la época más fría, permaneciendo latente bajo el suelo parte del tallo y la raíz que rebrotará en primavera. Algunas especies poseen la base de las ramas más antiguas algo lignificadas.

Indumento. La pubescencia del tallo, hojas y piezas florales es un carácter muy relevante. Puede haber dos tipos de pelos: glandulares y no glandulares (Fig. 1A). Los primeros tienen la cabezuela 1- a 2- o multicelular, la célula del cuello y la célula de pie breve o no, dando origen a pelos glandulares cortos o largos (Fig. 1B). Los pelos no glandulares son unicelulares, terminados en un extremo agudo (Fig. 1D), con una pared cuticular gruesa, lisa o muchas veces con ornamentaciones o con puntuaciones. La base es más o menos dilatada y está formada por 3–5 células a veces algo sobreelevadas de la superficie del órgano (Fig. 1C), muchas veces estas células poseen cistolitos (Robert, 1912; Botta, 1993). El tipo de pilosidad: híspida, estrigosa, escabrosa, hirsuta, pilosa, vilosa, pubérula o tomentosa; la disposición: pelos adpresos (Fig. 1D) o patentes (Fig. 1A); y la distribución de los pelos no glandulares son caracteres constantes dentro de cada especie, si bien la densidad del indumento es muy variable intraespecíficamente. La fauce de la corola presenta, a veces, pelos moniliformes.

Hojas. Las hojas pueden ser pecioladas, como en *Verbena lobata* Vell., o *Verbena caniuensis* Moldenke, o sésiles, como ocurre generalmente en las especies de la serie *Pachystachyae*. En este último caso según la forma de la base de la hoja y su inserción en el tallo se distinguen 4 tipos de bases foliares: cuneada (Fig. 1E), cuando los márgenes en la base forman un ángulo menor a 90°; redondeada (Fig. 1F), cuando los márgenes en la base forman un ángulo de más de 90°; amplexicaule (Fig. 1G), cuando los márgenes basales







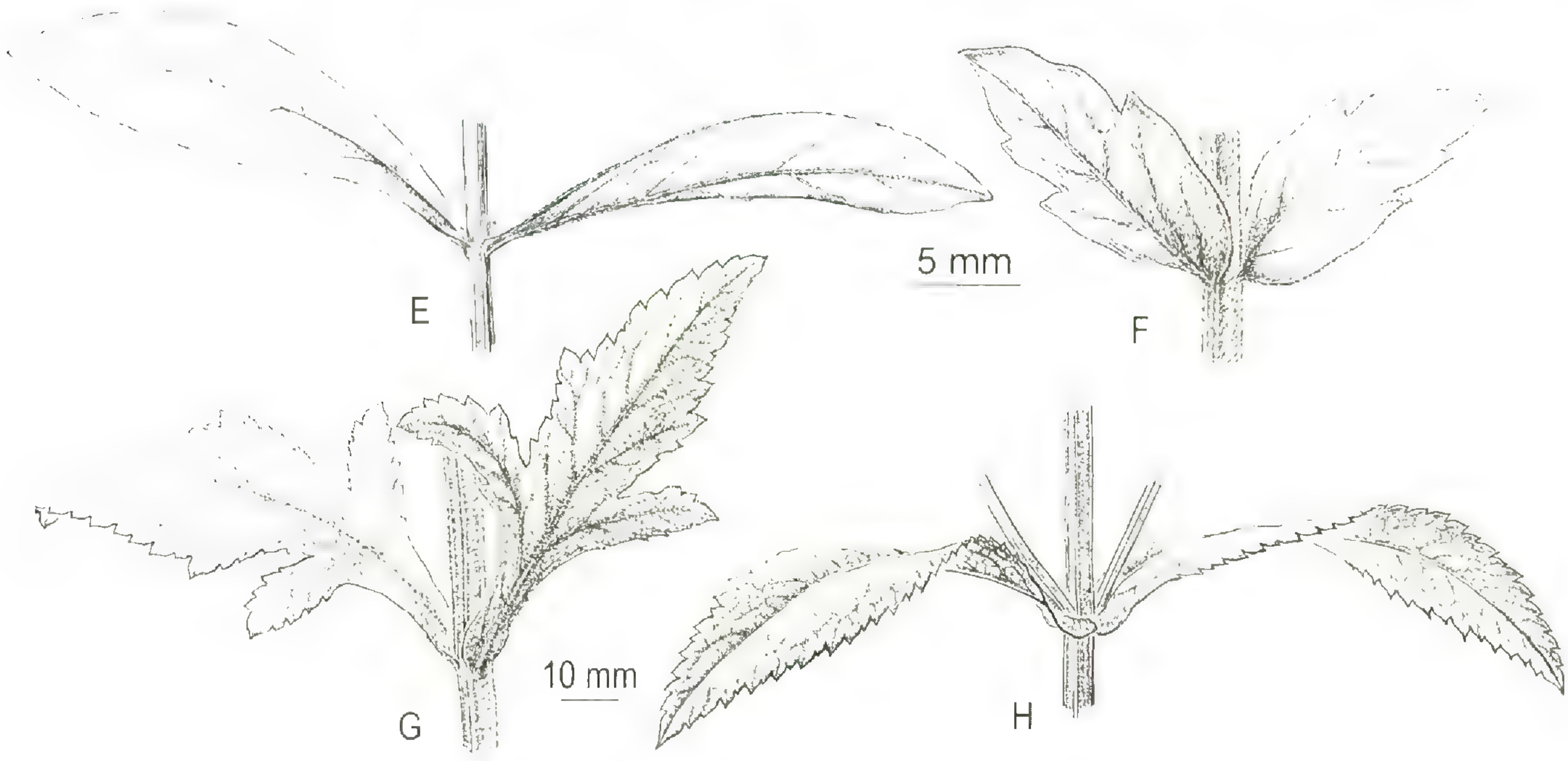


Figura 1. A–D. Pubescencia en *Verbena*. —A. Pelos glandulares y no glandulares en bráctea floral de *V. bonariensis* var. *conglomerata*. —B. Detalle de pelo glandular con pie largo en bráctea floral de *V. bonariensis* var. *conglomerata*. —C. Detalle de pelo no glandular con base dilatada, en superficie adaxial de hoja de *V. bonariensis* var. *bonariensis*. —D. Pelos no glandulares, unicelulares, terminados en extremo agudo, en superficie adaxial de hoja de *V. bonariensis* var. *bonariensis*. E–H. Base foliar. —E. Cuneada. —F. Redondeada. —G. Amplexicaule. —H. Auriculada. A, B: de *Guaglianone et al. 3273* (SI); C, D: de *Olmstead et al. 2004-144* (SI); E: *V. montevidensis* de *Herter 269* (SI); F: *V. sagittalis* de *Hagelund 7659* (SI); G: *V. hispida* de *Venturi 525* (SI); H: *V. bonariensis* de *Boffa s.n.* (SI 25870).

abrazan el tallo; y auriculada (Fig. 1H), cuando la base foliar presenta proyecciones pequeñas o grandes, redondeadas. Otra variación que se encuentra en los taxones de este serie es el tipo de ápice, agudo u obtuso, la incisión de los márgenes: entero, dentado o serrado, en toda la lámina o solo en porción distal de la lámina; y la integridad o no de la lámina: entera, lobulada, partida, sectada. Estos caracteres foliares de la forma y división de la lámina, a pesar de su variabilidad, son útiles para diferenciar taxones a nivel específico.

Arquitectura de la inflorescencia. De acuerdo con el trabajo de Martínez et al. (1996), *Verbena* posee inflorescencias de tipo politélico, lo cual significa que es de tipo abierto; formadas generalmente por pleiobotrios (más de una florescencia) heterotéticos (con florescencia principal), constituídos por la florescencia principal (FP) y una zona de enriquecimiento (ZE) frondosa o bracteosa, con paracladios simples o trímeros (PC), formados por la florescencia central y dos florescencias laterales; o paracladios ramificados o multímeros (PC′), de 1 a 2 ó más órdenes de ramificación (Fig. 2A, B). Cada florescencia es generalmente multiflora, laxa o densa según la disposición de las flores en la misma, sin bractéolas florales, de maduración acrópeta. El raquis de la florescencia contraído en la antesis, se alarga durante la fructificación, este alargamiento puede ser marcado o no. El entrenudo basal (EB) de la florescencia puede ser desarrollado o no desarrollado (florescencia sésil). Se define como hipotagma todos los elementos de un paracladio a excepción de la florescencia principal y del entrenudo basal. La variación en el grado de ramificación y desarrollo del hipotagma es la base de la diversidad morfológica de las inflorescencias de *Verbena*. Existen dos modelos básicos dentro de la serie *Pachystachyae*. Inflorescencias con uno o varios pares de paracladios distales simples o dicasialmente ramificados aglomerados alrededor de la florescencia principal, del aspecto corimbiforme y formando una unidad más o menos segregada de los paracladios basales (Fig. 2B), como ocurre en *V. alata* Otto ex Sweet, *V. bonariensis*, *V. ovata* Cham. y *V. hirta* Spreng.; o inflorescencias donde cada florescencia está subtendida por un pedúnculo de longitud variable lo cual impide que se aglomeren y el aspecto general es paniculiforme (Fig. 2A), como por ejemplo en *V. litoralis* y el resto de las especies.

Flor. Las flores son hermafroditas, generalmente pequeñas, menores de 10(–12) mm. Las brácteas florales poseen los márgenes subglabros a ligeramente pubérulos hasta ciliados. En cuanto a la longitud pueden ser de igual, menor o de mayor longitud que el cáliz. El cáliz es tubuloso y presenta 5 dientes subiguales breves o largos (se considera largo si el diente es igual o mayor a 1 mm), de forma triangular o subulada. La corola es generalmente infundibuliforme (hipocraterimorfa únicamente en *Verbena rigida*), apenas mayor en longitud que el cáliz o excediéndolo en gran medida, externamente pubescente o glabra, la fauce con pelos moniliformes, vilosa o glabra. En las especies de *Verbena* los tubos corolinos son de aproximadamente 2–7 mm, llegando excepcionalmente a 12 mm como ocurre a veces en *V. rigida*, *V. ovata* o *V. intercedens* Briq., los lóbulos se hallan poco desarrollados, excepto en *V. rigida*, donde suelen ser conspicuos. El color es azul, celeste, violáceo, lila, rojo, purpúreo, rosa o blanco. El androceo por lo general se ubica hacia la mitad superior del tubo corolino y el par superior de estambres no posee apéndices conectivales en las anteras. El gineceo posee estilo filiforme breve, siempre menor a tres veces la longitud del ovario.

Polen. Los granos de polen son tricolpados sin ornamentaciones notables, de simetría radial, isopolares, triangulares en vista polar, elípticos en vista ecuatorial (Raj, 1983).

Fruto. El cáliz fructífero envuelve el fruto y es de igual longitud que éste, no acrescente a la madurez, con dientes no conniventes. La dehiscencia del cáliz se produce en general a través de una línea longitudinal que permite la dispersión de las clusas maduras (Strasburger et al., 1974: 648). Los frutos son esquizocarpos de color castaño, cuadrangulares, que se separan en 4 clusas de sección trígona, de entre 1–3 mm de longitud. El dorso puede ser practicamente liso, como en *Verbena bonariensis*, o reticulado en su porción apical, abarcando desde un tercio de la superfice hasta más de la mitad, el resto es estriado; el grado de marcación del retículo o de las estrías es variable. La cara comisural de la clusa puede ser: papilosa; verrucosa, cuando estas papilas son poco protuberantes; o muricada cuando no hay papilas y pareciera formarse un entramado de excrecencias poco protuberantes (véase Barber, 1982: figs. 3, 4).

Fenómeno de malformación. Es frecuente encontrar plantas con una proliferación inusual de las florescencias, surgiendo de todos los nudos, incluso en los pedúnculos mismos. Las florescencias suelen ser más breves que la longitud que usualmente tienen, y muchas veces más anchas. La anomalía en cuanto a la posición y morfología de las mismas se acompaña por la presencia de flores con piezas atrofiadas. Por ejemplo ausencia de cáliz y corola, o corola muy breve y cáliz superándola. Esta malformación se ha encontrado en ejemplares de especies como *Verbena bonariensis* (*Puyssegur s.n.*, SI 3793!) y *V. intermedia* Gillies &

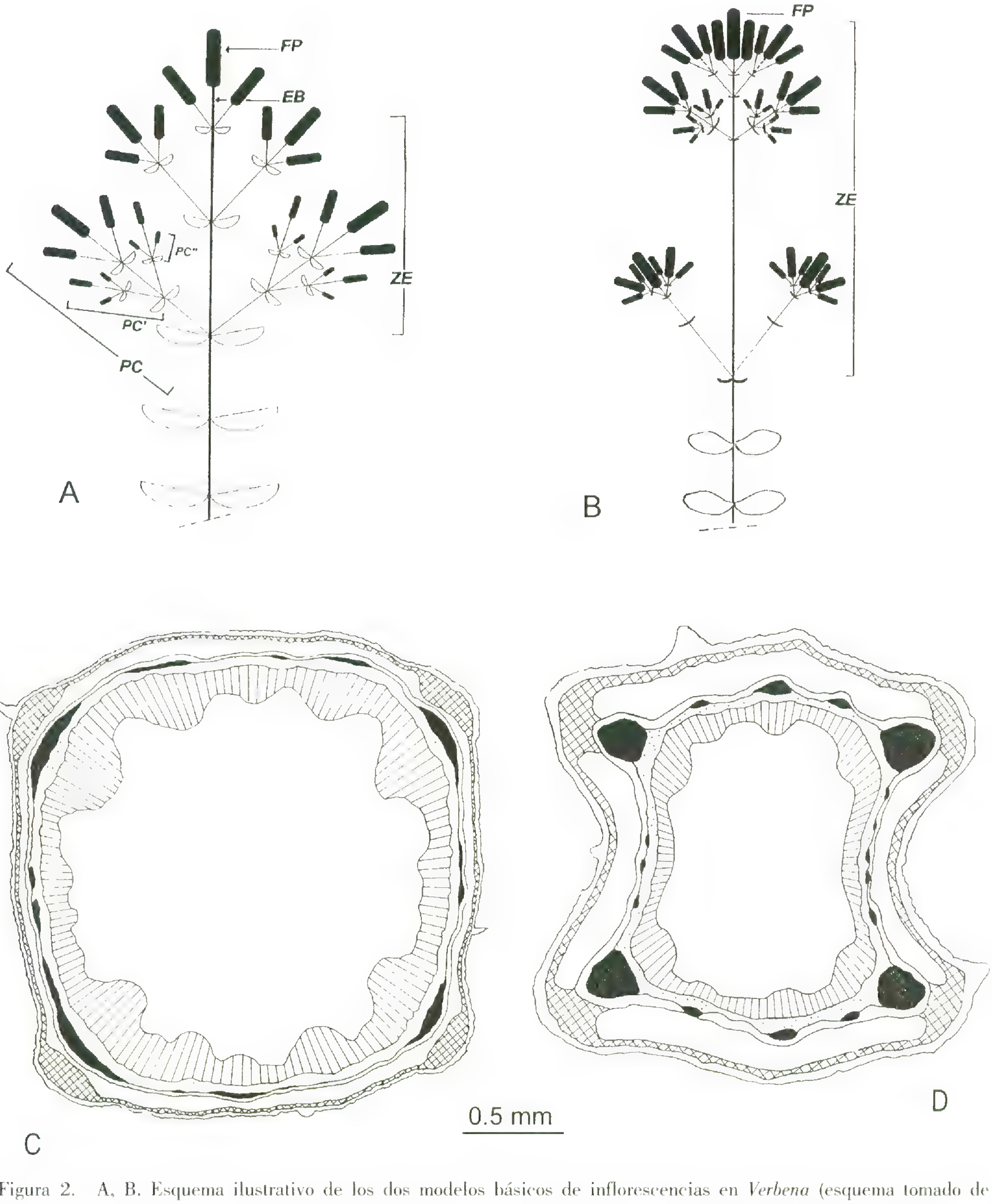


Figura 2. A, B. Esquema ilustrativo de los dos modelos básicos de inflorescencias en *Verbena* (esquema tomado de Martínez et al., 1996). —A. Tipo paniculiforme. —B. Tipo corimbiforme. FP = florescencia principal, EB = entrenudo basal a FP, ZE = zona de enriquecimiento, PC = paracladio. C, D. Esquema de los transcortes de tallo joven en *Verbena* (esquema tomado de Botta, 1989). —C. Tipo A con parénquima cortical continuo. —D. Tipo B con parénquima cortical discontinuo. (Se utilizaron los signos propuestos por Metcalfe y Chalk (1950) para diferenciar los tejidos.)

Hook. ex Hook. (*Burkart 26036*, SI!; *Bermdt 5160*, SI!). El nombre *V. carollata* Briq. se basa en un tipo que corresponde a un ejemplar de *V. intermedia* deformado (véase discusión bajo *V. intermedia*).

ANATOMÍA

Los tallos de *Verbena* son de sección tetragonal, pero algunas especies pueden presentar tallos ligeramente poligonales o circulares, principalmente en la base (ej. *V. ephedroides* Cham., *V. hirta*). El colénquima es de tipo angular y se dispone subepidérmicamente, generalmente de forma continua a lo largo de todo el perímetro del tallo. Puede estar engrosado en los 4 ángulos o en más de 4, o no engrosado; lo cual se relaciona con el tipo de sección del tallo, tetragonal, poligonal o circular. El esclerénquima puede estar formando 4 columnas muy

robustas, en los ángulos del tallo, o formando varios haces de similar diámetro sin relación con los ángulos del tallo. El parénquima cortical puede estar formando un anillo continuo, o discontinuo en al menos alguno de los ángulos, o discontinuo en todos los ángulos interrumpido por paquetes de esclerénquima. Entre el parénquima clorofiliano cortical hacia el exterior y los haces de esclerénquima y/o el floema hacia el centro se dispone una capa de células grandes, oblongas, que presentan bandas de Caspary, lo cual constituiría una endodermis caulinar. Se han encontrado dos tipos básicos de anatomías caulinares. Tipo A (Fig. 2C): con parénquima clorofiliano de disposición continua a lo largo de toda la circunferencia, no interrumpiéndose en los ángulos del tallo por presencia de paquetes de esclerénquima. Tipo B (Fig. 2D): con parénquima clorofiliano interrumpido en los ángulos por gruesas columnas de esclerénquima que se juntan con el colénquima angular.

Fenología

Las especies de *Verbena* poseen una floración prolongada, desde mediados de octubre hasta mediados de abril. Se ha observado que en ejemplares que crecen en zonas templado-cálidas, la floración puede comenzar desde el principio de la primavera y prolongarse hasta comenzado el invierno. Dado que la maduración de las florescencias es acrópeta, se encuentran frecuentemente flores y frutos en un mismo ejemplar, hacia el ápice aún hay flores y en la base ya hay frutos. Por lo tanto, a partir de diciembre pueden encontrarse frutos, si bien, obviamente será hacia fines de la estación estival que estos prevalecerán.

Tratamiento Taxonómico

Verbena L., Sp. Pl. 1: 18. 1753. TIPO: *Verbena officinalis* L. (lectotipo, designado por Britton & Brown (1913: 94)).

Obletia Le Monn. ex Rozier, Intr. Obs. Phys. Hist. Nat. 1: 367. 1771. TIPO: *Obletia verbenalacaea* Le Monn. ex Rozier.

Billardiera Moench, Methodus: 369. 1794, nom. illeg., non *Billardiera* Sm. 1753, non *Billardiera* Vahl, 1796. TIPO: *Billardiera explanata* Moench.

Verbenella Spach, Hist. Nat. Veg. 9: 237. 1838. TIPO: *Verbenella chamaedryfolia* (Juss.) Spach. (= *Verbena chamaedryfolia* Juss.).

Uwarowia Bunge, Bull. Sci. Acad. Imp. Sci. Saint-Pétersbourg 7: 278. 1840. TIPO: *Uwarowia chrysanthemifolia* Bunge.

Helleranthus Small, Fl. S.E. U.S. ed. 1: 1011. 1903. TIPO: *Verbena quadrangulatus* Heller (= *Helleranthus quadrangulatus* (Heller) Small).

Hierbas anuales o perennes, o sufrútices, muchas veces hemicriptófitos, de hábito erecto, decumbente a procumbente; tallos tetrágonos a poligonales, a veces algo cilíndricos en la base, desde glabros a pubescentes con pelos adpresos a patentes, simples o glandulares; braquiblastos desarrollados o no. *Hojas* opuestas decusadas, lámina desarrollada, entera, lobada, partida, o sectada; márgenes muy variados, generalmente serrado-dentados a irregularmente crenados, base sésil o peciolada; ápice agudo u obtuso; pubescencia en ambas caras pero menos densa en la cara adaxial siendo a veces subglabras. *Inflorescencia* politélica formada por pleiobotrios heterotéticos, constituídos por una florescencia principal siempre presente y una zona de enriquecimiento con paracladios simples o ramificados, sinflorescencia bracteosa o frondosa, florescencias cilíndricas densas o filiformes laxas, pedunculadas a sésiles, multifloras, flores densamente imbricadas o dispuestas laxamente, de maduración acrópeta, sin bractéolas florales. *Flores* perfectas, en general pequeñas, sésiles, a veces sobre pedicelos breves, corola levemente zigomórfica, bráctea floral presente, cáliz cilíndrico-tubular, 5-angulado, con 5 dientes desiguales; corola infundibuliforme o hipocraterimorfa, de color blanco, azul, rojo y sus diferentes gamas, con tubo cilíndrico recto o a veces algo curvado, con o sin pubescencia en la fauce y en la garganta de la misma, con 5 lóbulos poco desarrollados, desiguales; estambres 4, didínamos, subsésiles, generalmente insertos en la mitad superior del tubo corolino, filamento muy breve o ausente; anteras ovoides, de dehiscencia longitudinal; ovario súpero, bi-carpelar, cada carpelo bi-locular con un óvulo por lóculo inserto en el borde del carpelo; estilo breve, menos de tres veces la longitud del ovario, base del estilo no ensanchada, de inserción apical, estigma generalmente bi-lobado, con un lóbulo algo redondeado y papiloso y el otro agudo y liso. *Fruto* esquizocárpico formado por 4 clusas uniseminadas, cilíndricas de sección subtrígona, ápice obtuso y base no angostada, de igual longitud o algo menor que el cáliz fructífero.

Distribución y Habitat

El género *Verbena* es de origen americano (Lewis & Oliver, 1961); actualmente se extiende por América tropical y templada, norte de África, Asia, Europa y en Australia; crece desde el nivel del mar hasta ca. 4000 m s.m. en la región andina de Ecuador, Bolivia y Perú. Existen tres grandes centros de diversidad específica (Sanders, 2001: 334), uno en zonas templado-cálidas del noreste Argentino y sur de Brasil, taxones principalmente pertenecientes a la serie *Pachystachyae*; el segundo en el sudoeste de Estados Unidos de América y norte de México, con algunas especies en la zona Pacífica y montañosa; el

Tabla 1. Subdivisiones del género *Verbena* serie *Pachystachyae*, con las especies pertenecientes a cada subserie estudiadas en el presente trabajo.

serie *Pachystachyae*	
I. subserie *Pachystachyae*	II. subserie *Pseudoracemosae*
Verbena alata Otto ex Sweet	*Verbena caniuensis* Moldenke
Verbena bonariensis L.*	*Verbena filicaulis* Schauer
Verbena ephedroides Cham.	*Verbena hirta* Spreng.*
Verbena glabrata Kunth	*Verbena lobata* Vell.*
Verbena hispida Ruiz & Pav.*	*Verbena subpetiolata* N. O'Leary
Verbena intercedens Briq.	
Verbena intermedia Gillies & Hook. ex Hook.	
Verbena lindbergii Moldenke	
Verbena litoralis Kunth*	
Verbena montevidensis Spreng.	
Verbena ovata Cham.	
Verbena rigida Spreng.	
Verbena sagittalis Cham.	

* Especies que poseen variedades.

tercero en la zona templada de este de Estados Unidos de América y sur de Canadá; estos dos últimos centros representados principalmente por taxones pertenecientes a la serie *Verbena*.

RELACIONES INFRAGENERICAS

La serie *Pachystachyae* agrupa 18 especies y cinco variedades. Se caracteriza por las florescencias cilíndricas, multifloras, con flores y frutos densamente imbricados a excepción de los nudos basales de la misma. Las florescencias poseen flores densamente imbricadas, se reunen en paracladios trímeros o multímeros, de aspecto paniculiforme o corimbiformes, o son solitarias. Sanders (2001) sugirió que esta serie sería monofilética y los caracteres en los que estaría basada serían apomórficos. Esta serie incluye parte de los taxones pertenecientes a los grupos informales denominados Bonariensis, Hastatae y Venosae de Small (1933).

Se observó un grupo de cinco especies morfológicamente diferentes del resto, que a su vez presentaban una anatomía caulinar común entre sí y distinta de la encontrada en las demás especies: disposición continua del parénquima clorofiliano caulinar. Por lo tanto, se fundó la subserie *Pseudoracemosae* que incluye estas 5 especies.

En la Tabla 1 se presenta la lista de especies de la serie *Pachystachyae*.

CLAVE PARA LAS SERIES DEL GENERO *VERBENA*

1a. Florescencias generalmente filiformes, laxas, hasta de 30 cm en la fructificación, flores distanciadas entre sí, frutos remotos a lo largo de la misma; plantas de origen norteamericano . . . . . serie *Verbena*

1b. Florescencias no filiformes, densas, breves o largas hasta de 20 cm en la fructificación, flores densamente imbricadas, frutos distanciados únicamente hacia la base de las mismas; plantas de origen sudamericano . . . . . serie *Pachystachyae*

2a. Flores densamente imbricadas en la florescencia; anatomía caulinar tipo B (excepto *V. rigida*): transcorte caulinar con disposición discontinua del parénquima clorofiliano, interrumpido por columnas de esclerénquima . . . . . subserie *Pachystachyae*

2b. Flores distanciadas 2 mm o más en la florescencia; anatomía caulinar tipo A: transcorte caulinar con disposición continua del parénquima clorofiliano . . . . subserie *Pseudoracemosae*

CLAVE PARA DIFERENCIAR LAS ESPECIES DE *VERBENA* SERIE *PACHYSTACHYAE*

1a. Hojas glabras o subglabras, con escasos pelos adpresos; las piezas florales con pelos no glandulares o si glandulares entonces muy breves.

2a. Sinflorescencia solo con ramificación de primer orden, hipotagma ausente, florescencias sésiles; brácteas florales con márgenes ciliados, cáliz con 5 dientes largos, subulados . . . . . 13. *V. sagittalis*

2b. Sinflorescencia con ramificación de primer o mayor orden, hipotagma presente, florescencias pedunculadas; brácteas florales con márgenes pilosos, cáliz con 5 dientes breves, triangulares.

3a. Hojas pecioladas, éste mayor a 0.5 mm.

1a. Florescencias solitarias; flores pediceladas . . . . . 14. *V. caniuensis*

4b. Florescencias reunidas en paracladios, sinflorescencia de aspecto piramidal; flores sésiles . . 17. *V. lobata*

3b. Hojas sésiles a subsésiles.

5a. Hojas basales de lámina trilobada a bipinnatisectada, las apicales a veces enteras . . . 15. *V. filicaulis*

5b. Hojas basales y apicales de lámina entera.

6a. Hojas de base subamplexicaule o base auriculada.

7a. Entrenudo basal de la florescencia principal poco o no desarrollado; florescencias laterales no superan la florescencia principal, cada florescencia con característico aspecto subcónico . . . . . 4. *V. glabrata*

7b. Entrenudo basal de la florescencia principal no desarrollado; florescencias laterales superan la florescencia principal o no, florescencias sin aspecto subcónico.

8a. Hojas basales con lámina elíptico-angosta o sublinear, márgenes lisos o apenas dentados con 1–3 dientes breves a cada lado en porción distal; nudos superiores con hojas de aspecto semejante, aunque reduciéndose en tamaño; generalmente inflorescencia laxa . . . . . 8. *V. lindbergii*

8b. Hojas basales con lámina elíptica, márgenes lisos o serrados en toda la lámina; nudos superiores con hojas triángulo-ovadas reducidas en tamaño hasta escuamiformes, teniendo muchas veces aspecto subáfilo, generalmente inflorescencia congesta . . . . . 1. *V. alata*

6b. Hojas de base cuneada.

9a. Plantas con hojas superiores ausentes o escamosas, las basales de lámina desarrollada, elíptico-angosta; florescencias de 0.6 cm de ancho . . . . . 3. *V. ephedroides*

9b. Plantas con hojas superiores reduciéndose en tamaño, siempre presentes, las basales de lámina muy variada, elíptica, ovada, oblonga; florescencias de 0.4–0.55 cm de ancho.

10a. Plantas de aspecto grácil, hasta de 1 m de altura; florescencias breves hasta de 6 cm en fructificación . . . . . 10. *V. montevidensis*

10b. Plantas de aspecto no grácil, hasta de 3 m de altura; florescencias hasta de 18 cm en fructificación . . . . . 9. *V. litoralis*

1b. Hojas pubescentes principalmente en el envés, con abundantes pelos adpresos o patentes; las piezas florales con pelos glandulares largos (excepto *V. ovata*).

11a. Hojas pecioladas, éste entre 0.5–1 mm . . . . . 17. *V. lobata*

11b. Hojas sésiles o sí subpecioladas éste menor a 0.5 mm.

12a. Sinflorescencia bracteosa, ramificaciones de segundo o mayor órden.

13a. Florescencias reunidas en paracladios multímeros aglomerados en torno a la florescencia principal, nunca superándola.

14a. Brácteas florales de tamaño mayor que el cáliz, márgenes pilosos; cáliz con pelos adpresos, no glandulares . . . . . 11. *V. ovata*

14b. Brácteas florales de tamaño menor o igual que el cáliz, márgenes ciliados; cáliz con pelos patentes, con o sin pelos glandulares . . . . . 2. *V. bonariensis*

13b. Florescencias reunidas en paracladios trímeros no aglomerados en torno a la florescencia principal, superándola.

15a. Hierbas erectas, robustas hasta de 3 m de altura.

16a. Flores de tubo corolino mayor de 0.7 cm; brácteas florales ovado-angostas, de 0.4–0.55 cm . . . . . 6. *V. intercedens*

16b. Flores de tubo corolino menor de 0.5 cm; brácteas florales ovadas, de (0.15–) 0.2–0.35(–0.4) cm . . . . . 9. *V. litoralis*

15b. Sufrútices o hierbas, erectas o decumbentes, subgráciles hasta de 1 m de altura.

17a. Hojas oblongo-angostas a elíptico-angostas, ápice agudo, las basales provistas de 2–3 pares de dientes opuestos o casi alternos en la mitad distal de la lámina, las superiores sublineares de márgenes liso, generalmente dispuestas de forma erecta, algo adpresas al tallo; corola infundibuliforme . . . . . 7. *V. intermedia*

17b. Hojas oblongas, elípticas u obovadas, no angostas, ápice agudo o subobtuso, márgenes marcada y uniformemente inciso-serrados, dientes agudos espaciados entre sí por senos cóncavos, nunca adpresas al tallo; corola hipocraterimorfa . . . . . 12. *V. rigida*

12b. Sinflorescencia frondosa, ramificaciones de primer orden.

18a. Florescencias de 1.5(–4) cm; clusas de 2–2.5 mm; hojas de base cuneada, subtruncada o redondeada.

19a. Plantas hasta de 30 cm altura, hojas de base subtruncada a redondeada, nunca cuneada . . . . . 18. *V. subpetiolata*

19b. Plantas mayores a 30 cm altura, hojas de base cuneada . . . . . 16. *V. hirta*

18b. Florescencias de 4–8(–15) cm; clusas de 1.5–2 mm; hojas de base subamplexicaule a cuneado-atenuada . . . . . 5. *V. hispida*

I. Verbena serie **Pachystachyae** subserie **Pachystachyae** Schauer, Prodr. 11: 546. 1847. TIPO: *Verbena bonariensis* L. (lectotipo, designado por Troncoso (1974: 311)).

La subserie *Pachystachyae* está conformada por 13 especies y tres variedades; agrupa todos aquellos taxones con florescencias con flores densamente imbricadas y con transcorte caulinar tipo B, con disposición del parénquima clorofiliano de manera discontinua (véase Fig. 2D), excepto en una especie: *Verbena rigida*, que presenta anatomía caulinar tipo A, pero comparte el resto de los caracteres de esta subserie.

La subserie *Pachystachyae* es estrictamente sudamericana con algunas especies naturalizadas en regiones templado-tropicales del resto del mundo. Existe un centro de alta diversidad en el noreste de Argentina (Entre Ríos, Corrientes y Misiones) y sur de Brasil (Rio Grande do Sul, Santa Catarina y Paraná) (Sanders, 2001).

1. Verbena alata Otto ex Sweet, Brit. Fl. Gard. 4: tab. 41. 1830. TIPO: Ilustración de Sweet, 1830, en Brit. Fl. Gard. 4: tab. 41 (lectotipo, designado por O'Leary (2006: 494)). Figura 3A, B.

Hierbas perennes, erectas, hasta de 1.5–2 m de altura, ramificadas desde la base, tallos marcadamente tetrágonos, subglabros, longitudinalmente estriados, alados en los ángulos, nudos notoriamente contraídos. *Hojas* de lámina entera, elíptica, las basales de (1–)4–4.5(–6) × 0.5–3 cm, ápice agudo, sésiles de base subamplexicaule, márgenes lisos a serrados en toda la lámina, subglabras con algunos pelos escabrosos en ambas superficies, rugosas al tacto; las apicales de lámina escasa, reducidas en tamaño, triángulo-ovadas de 1 cm, hasta escuamiformes, muchas veces aspecto subáfilo hacia el ápice. *Florescencias* terminales breves y densas, compactas de 1.5(–3.5) × (0.45–)0.55(–0.6) cm, pedúnculos breves, hipotagma presente, agrupadas en paracladios trímeros aglomerados alrededor de la florescencia principal, superándola o no, ésta sésil o brevemente pedunculada, ramificaciones de segundo o mayor orden, sinflorescencia bracteosa, aspecto corimbiforme; brácteas florales ovadas de 0.2–0.25(–0.3) cm, ápice acuminado, ligeramente estrigosas, los márgenes esparcidamente pilosos. *Flores* sésiles de corola color roja, violácea o blanca; cáliz de (0.22–)0.25–0.3(–0.35) cm, uniformemente piloso a veces más evidente sobre las costillas, 5 dientes triangulares breves de ápice agudo; corola infundibuliforme, tubo corolino de 0.45–0.55 cm, pubescente externamente en la garganta e internamente en la fauce; estambres insertos hacia la mitad de la longitud del tubo corolino. Clusas de 2 mm, dorso reticulado en el tercio apical, el resto estriado, cara comisural papilosa. Anatomía caulinar tipo B.

Distribución y ecología. Endémica del sur de Brasil en los estados de Rio Grande do Sul, Santa Catarina y Paraná. Habita en campos húmedos y matorrales, en los bordes de cursos de agua.

Observaciones. *Verbena alata* es fácilmente reconocible por tener los tallos alados a lo largo de las aristas de los entrenudos, contrayéndose en los nudos. Esta especie es morfológicamente afin a *V. lindbergii* y *V. ephedroides*, compartiendo con éstas la escasa pubescencia y ausencia de pelos glandulares. *V. lindbergii* se diferencia por las hojas de lámina elíptico-angosta o sublinear y los márgenes apenas dentados en la porción distal, con hojas presentes inclusive en los nudos superiores; *V. ephedroides* se diferencia por las hojas de base cuneada.

Tipificaciones y nomenclatura. *Verbena alata* Chamisso (1832: 258) es un nombre ilegítimo (Art. 53.1, Greuter et al., 2000) por homónimo posterior de *V. alata* Otto ex Sweet de 1830, taxón del cual sería sinónimo. Moldenke (1961: 146) es el primer autor en reconocer que el taxón de Sweet posee prioridad sobre el de Chamisso.

Material adicional examinado. BRASIL. **Paraná:** Bocaiuva do Sul, *Hatschbach 20239* (SI). **Rio Grande do Sul:** Taimbesinho, 20 Feb. 1953, *Rambo s.n.* (SI ex PACA 53969); Torres, *Burkart 25163* (SI). **Santa Catarina:** Stellen bei Tubarão, *E. Ule 1068* (CORD); Bom Retiro, *Reitz c1965* (SI); San José, *Reitz & Klein 10205* (SI); Lajes, *Smith & Klein 8065* (SI); Porto União, *Reitz & Klein 11673* (SI).

2. Verbena bonariensis L., Sp. Pl. 1: 20. 1753. TIPO: "*V. bonariensis* 9 LINN 35.11" (lectotipo, designado por Yeo (1990: 105), LINN no visto; isotipo, LINN foto SI!).

Hierbas perennes, erectas, hasta de 0.5–2.5 m de altura; tallos tetrágonos, leñosos en la base de ejemplares adultos, con pubescencia escabrosa a híspida principalmente en las aristas. *Hojas* de lámina entera, ovado-elípticas o sublineares, de (3–)5–10 (–18) × (0.5–)1–3(–6) cm, ápice agudo, sésiles de base subauriculada a auriculada, los márgenes irregularmente serrados; con pubescencia tomentosa a hirsuto-escabrosa sobre las venas de la cara abaxial, pubescencia hirsuto-estrigosa en cara adaxial. *Florescencias* terminales, subcilíndricas, subcapitadas, densas, de 0.8–6 × 0.5–0.6 cm, reunidas en paracladios multímeros aglomerados en torno a la florescencia principal sésil, las florescencias laterales brevemente pedunculadas, nunca superando a la principal, sinflorescencia bracteosa con ramificaciones de segundo orden o mayor, hipotagmas

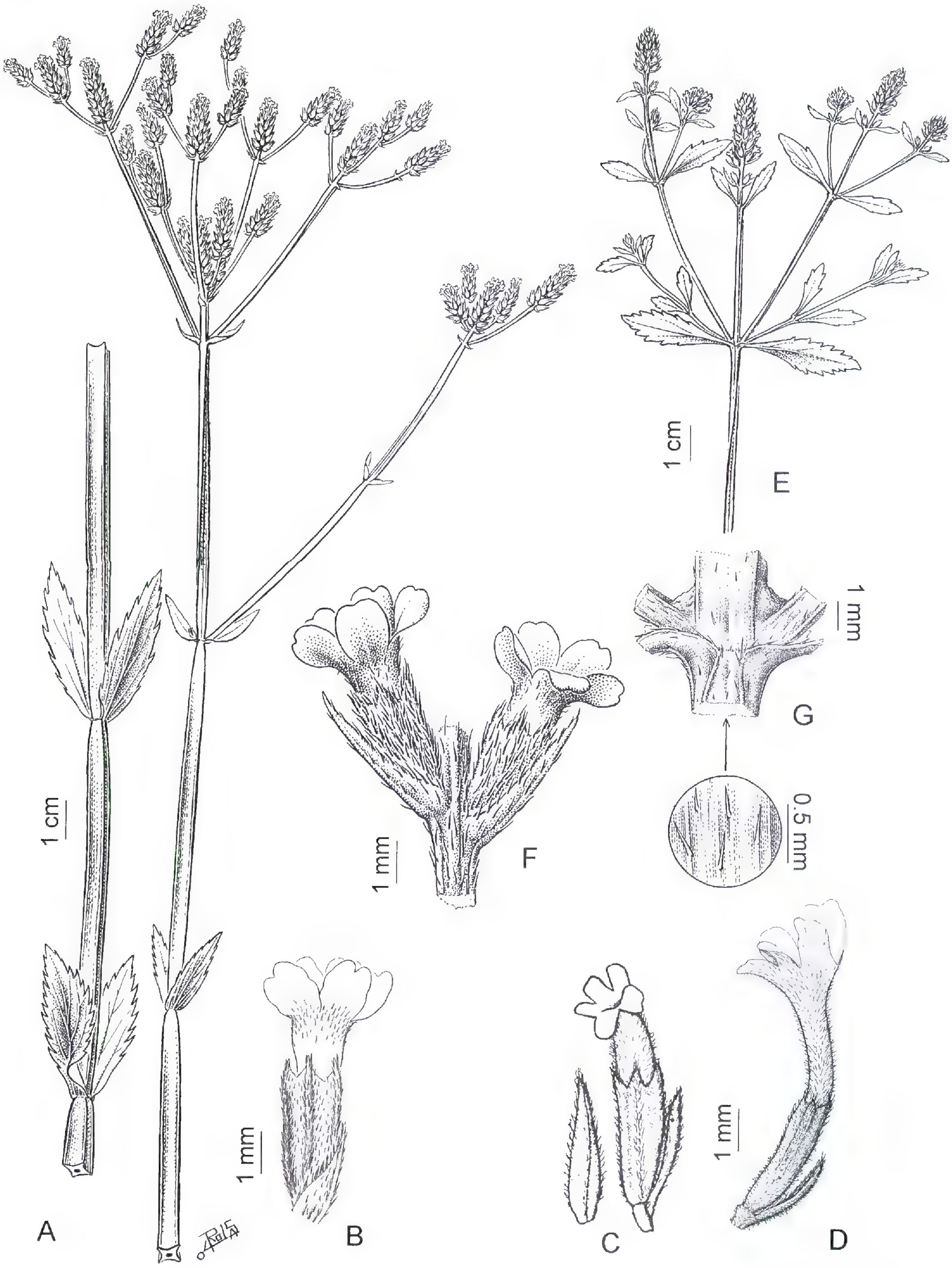


Figura 3. A, B. *Verbena alata.* —A. Aspecto general de rama con inflorescencia. —B. Flor. C, D. *Verbena bonariensis.* —C. Flor de *V. bonarienisis* var. *bonariensis.* —D. Flor de *V. bonariensis* var. *conglomerata.* E–G. *Verbena hispida* var. *obovata.* —E. Porción apical rama con inflorescencia. —F. Detalle flores. —G. Detalle nudo con escasa pubescencia de pelos adpresos. A, B: de *Burkart 25163* (SI); C: de *Cabrera 8496* (SI); D: de *Burkart 27357* (SI); E, G: de *Solomon 12759A* (SI); F: de *Krach 7793* (SI).

desarrollados; brácteas florales ovadas o elíptico-angostas de 0.3(–0.4) cm, los márgenes ciliados. *Flores* sésiles, corola color purpúreo, violáceo, lila o blanco; cáliz de 0.25–0.3(–0.4) cm, con pelos patentes, con o sin pelos glandulares, con 5 dientes triangulares, de ápice agudo; corola infundibuliforme, tubo corolino cilíndrico angosto, de (0.35–) 0.45–0.6(–0.7) cm, apenas exerto del cáliz o casi el doble de la longitud del mismo, pubescencia vilosa exteriormente, fauce glabra, limbo poco desarrollado; estambres insertos sobre la mitad de la longitud del tubo corolino. Clusas de 1.2–2 mm, de dorso suavemente reticulado en porción distal, el resto ligeramente estriado, cara comisural muricado-verrucosa. Anatomía caulinar tipo B.

Número cromosómico. $n = 6, 7$ (Kumar & Dutt, 1989), $n = 14$ (Vasudevan, 1975), $2n = 28$ (Dermen, 1936; Noack, 1937; Chaw et al., 1986).

Observaciones. *Verbena elongata* Salisb., Prodr. Stirp. Chap. Allerton: 71. 1796, nom. illeg. superfl. *Verbena trichotoma* Moench, Suppl. Meth.: 131. 1802, nom. illeg. superfl. Ambos nombres se basan en el ejemplar tipo de *V. bonariensis* por lo cual son nombres supérfluos (Art. 52, Greuter et al., 2000).

CLAVE DE LAS VARIEDADES DE *VERBENA BONARIENSIS*

2a. Tubo corolino raramente mayor de 0.5 cm; brácteas florales de ápice agudo, generalmente de igual o mayor longitud que el cáliz; pedúnculos de las florescencias generalmente sin pelos glandulares, ocasionalmente presentes en piezas florales; hojas de lámina ovado-elíptica . . . . . 2a. var. *bonariensis*

2b. Tubo corolino generalmente mayor de 0.5 cm, casi el doble en longitud que el cáliz; brácteas florales de ápice obtuso a subobtuso, generalmente de menor longitud que el cáliz; pedúnculos de las florescencias y piezas florales con pelos glandulares; hojas generalmente de lámina sublinear . . . . . 2b. var. *conglomerata*

2a. Verbena bonariensis var. **bonariensis** L.

Figura 3C.

Verbena bonariensis f. *albiflora* Moldenke, Phytologia 3: 117. 1949. Syn. nov. TIPO: Argentina. Entre Ríos: Dpto. Gualeguay, Isla El Cavadito, Ene. 1947, *A. V. de Sota 82* (holotipo, NY no visto; isotipo, NY foto SI!).

Verbena incompta P. W. Michael, Telopea 6: 181. 1995. TIPO: Australia. Victoria: Bright-Tawonga Rd., 25 Mar. 1964, *A. C. Smith* 64/64 (holotipo, MEL no visto; isotipos, MEL foto SI!, NSW no visto).

Hojas de lámina ovado-elíptica, de base subauriculada a auriculada, márgenes irregularmente serrados hacia la base de la lámina, cartácea, venación poco evidente en la cara adaxial. Florescencias de 4 × 0.5 cm, en fructificación llegando a 6 cm; densas, agregadas en sinflorescencias congestas, los pedúnculos generalmente sin pelos glandulares, las piezas florales ocasionalmente con pelos glandulares cortos; brácteas florales ovadas de igual longitud que el cáliz o apenas superándolo, de ápice agudo; tubo corolino breve hasta de 0.5 cm, apenas superando en longitud al cáliz.

Iconografía. Gleason, 1952: 128; Marzocca, 1963: 323, fig. 61A; Troncoso, 1965: 130, fig. 45a–d; Lombardo, 1983: 54, lam. 19(1); Chaw et al., 1986: 126, fig. 1a–h; Yeo, 1990: 106, fig. 2a–g; Munir, 2002: 32, fig. 1a -n; De Marzi, 2006: 85 (foto).

Distribución y ecología. En Argentina es abundante en gran parte del territorio templado-cálido como la Mesopotamia, el noroeste y el centro del país. Se han encontrado ejemplares en regiones árido-secas de la provincia de Río Negro y en la región cuyana. También se distribuye por el sur de Bolivia en el departamento de Tarija, y en Paraguay, Uruguay y sur de Brasil. Ha sido introducida en otras partes del mundo como norte y centro América (Perry, 1933), regiones de Europa (Francia), África (Munir, 2002), Asia tropical (citada para Taiwan como maleza; Chaw et al., 1986) y Australia y Nueva Zelanda (Munir, 2002).

Constituye una planta invasora de campos de cultivo, a veces agresiva; se la encuentra en suelos modificados, potreros, terraplenes, baldíos, rastrojos, donde el suelo es bien drenado, húmedo y fértil o también en suelo algo arcilloso. Se propaga por semillas fácilmente a lo largo de ríos y bordes de canaletas inundadas. Moldenke (1983) señala que es una planta usada como nectarífera por las abejas. Chaw et al. (1986) mencionan que aparentemente sería de fecundación auto-compatible.

Observaciones. *Verbena bonariensis* se distingue fácilmente por sus inflorescencias de aspecto corimboso. Este carácter la asemeja a *V. ovata*, diferenciándose esta última por la pubescencia escasa de pelos adpresos en las piezas florales, las brácteas florales sobrepasando largamente el cáliz y hojas usualmente ovadas.

La presencia de base foliar auriculada es otro carácter que distingue a *Verbena bonariensis*, aunque lo comparte también con otros taxones, como *V. rigida*, *V. intercedens* y *V. intermedia*, pero en estas tres especies las florescencias se disponen en paracladios trímeros laxos no aglomerados en torno a la florescencia principal y presentan pelos glandulares distribuidos en las piezas florales.

Verbena bonariensis es un taxón muy variable en cuanto a las dimensiones de las flores y la

pubescencia. Michael (1995) establece una nueva especie, *V. incompta*, distinguiéndola de *V. bonariensis* por tener principalmente flores de menor tamaño y ausencia de pelos glandulares en las piezas florales. Sin embargo estos caracteres no permiten diferenciarla de *V. bonariensis* s. str. como se observa del análisis de los ejemplares (ej. *Montes 3334*, SI!; *Rambo 38179*, SI!).

Se han encontrado ejemplares con florescencias deformadas, posiblemente por la acción de un insecto (ej. *Rojas 8666a*, SI!), produciendo un crecimiento desproporcionado de las florescencias (véase bajo *Verbena intermedia*). Por otro lado, se han observado ejemplares que poseen la base de los tallos leñosa evidenciando varios años de crecimiento (ej. *Correa 10117*, SI!).

Moldenke (1949b: 117) consideró que el color de las flores de las verbenas constituía un carácter de relevancia taxonómica, por lo cual fundó la forma *albiflora* para abarcar los ejemplares con flores blancas. Sin embargo en *Verbena* el color de las flores es muy variable, por lo cual se considera que este carácter no posee valor taxonómico.

Existen otros taxones infraespecíficos bajo *Verbena bonariensis*, pero se sinonimizaron a otras especies de *Verbena* (véase sinonimia bajo *V. intercedens*, *V. intermedia*, *V. litoralis* y *V. rigida*).

Nombre vulgar. "Yerba de fierro", "verbena amarga", "verbena encañutada", "yerba de acero", "yerbón" en Argentina (De la Peña & Pensiero, 2004: 286).

Material adicional examinado. ARGENTINA. **Buenos Aires:** Pdo. Azul, s. loc., 1909, *Puyssegur s.n.* (SI 3793); Pdo. Berisso, Los Talas, 20 Feb. 1945, *Boffa s.n.* (SI 25870); Pdo. Vte. López, Olivos, vías del tren, *Botta 147* (SI); Pdo. Gral. Lavalle, Cari Lauquen, Talares, *Cabrera 8496* (LP, SI). **Catamarca:** Dpto. Andalgalá, Andalgalá, *Jørgensen 1295* (BAB, SI). **Chaco:** Dpto. San Fernando, isla Soto, *Schinini 16105* (CTES, SI). **Córdoba:** Dpto. San Javier, San Javier, *Castellanos 10833* (BA). **Corrientes:** Dpto. San Miguel, 12 km de Caa-Catí, *Ahumada et al. 2337* (CTES, SI). **Entre Ríos:** Dpto. Concordia, Estac. Agron., *Burkart 950* (SI); Calabacilla, ca. 10–15 km S Concordia, *Olmstead et al. 2004-104* (SI, US, WTU). **Formosa:** Dpto. Formosa, ca. Pirané, *Fortunato et al. 6087* (BAB, CTES); Tres Lagunas, Pta. Porá, *Rojas 8666a* (SI). **Jujuy:** Dpto. Dr. M. Belgrano, Lag. de Yala, *Cabrera et al. 21335* (LP, SI). **La Pampa:** Gral. Pico, *Williamson 2900* (BAB). **Mendoza:** Dpto. Las Heras, *King 137* (LP). **Misiones:** Dpto. Guaraní, ruta 13, *Múlgura et al. 3028* (SI). **Río Negro:** Dpto. Conesa, ruta 250, *Crespo & Calieres 2530* (BAB); Dep. A. Alsina, 100 km de pte. Viedma-Patagones, *Correa 10117* (SI). **San Luis:** Pancanta, *Castellanos s.n.* (BA 25/933). **Santa Fe:** Dpto. Castellanos, ruta 34, ca. INTA, *Pensiero 1137* (SI). **San Juan:** Dpto. Barreal, cuesta de Tambolar, *Sayago 2454* (SI). **Tucumán:** Dpto. Tafí, desde Las Carreras a El Molar, *Cialdella et al. 77* (CTES, SI). BOLIVIA. **Tarija:** Rincón de la Victoria, *Krapovickas et al. 18873* (SI). BRASIL. **Minas Gerais:** Poços de Caldas, *Krapovickas & Cristóbal 35393* (CTES). **Paraná:** Ponta Grossa, L. Dourada, *Dombrowski 9103* (SI). **Rio Grande do Sul:** s. loc., *Rambo 38179* (SI), ruta de San Gabriel a Porto Alegre, peaje, *O'Leary 507* (SI). **Santa Catarina:** Itajaí, Capoeira, *Klein 2518* (SI). **São Paulo:** ca. Ponte do Yaguaré, *Hoehne 15152* (CTES). PARAGUAY. **Alto Paraguay:** Pt. Casado, *Rojas 1892* (AS, SI). **Caazapá:** Tavaí, *Basualdo 2070* (FCQ). **Central:** San Lorenzo, Campo Gde., *Pavetti & Rojas s.n.* (AS). **Guairá:** Villarica, *Jørgensen 3768* (BA, LP, SI). **Itapúa:** Pto. Cantera, *Montes 3334* (SI), Yacyretá, *Zardini & Gamarra 59238* (AS, MO, SI). **Misiones:** Santiago, *Pedersen 4341* (CTES, SI). **Ñeembucú:** Ea. Espinillo, *Schulz 7808* (CTES). **Paraguarí:** Pque. Nac. Ibicuí, *Zardini & Velásquez 16051* (PY). **Pte. Hayes:** Km 60, *Mereles 1772* (FCQ, SI). **San Pedro:** Pto. Antequera, *Soria 5530* (FCQ). URUGUAY. **Montevideo:** Miguelete, *Herter* 268 (SI).

FRANCIA. Ile de la Réunion, St. Denis, *Lourteig 2449* (cultivada) (SI).

NUEVA ZELANDA. North Island, Auckland Co. Parnell, Shore Rd., *Goulding 713* (introducida) (CTES).

2b. Verbena bonariensis var. **conglomerata** Briq., Ark. Bot. 2(10): 11, tab. 3B. 1904. TIPO: Brasil. Rio Grande do Sul: Porto Alegre, "in pascuis siccis arenosis", 12 Oct. 1892, *C. A. M. Lindman A 473* (lectotipo, designado por Munir (2002: 36), S no visto; isotipo, S foto SI!). Figura 3D.

Verbena inamoena Briq., Bull. Herb. Boissier, sér. 2(4): 1058. 1904. Syn. nov. TIPO: Paraguay. Tobaty, Ago. 1899, *E. Hassler 3164* (holotipo, G no visto; isotipo, SI!).

Hojas de lámina elíptico-angosta a sublinear, de base auriculada, márgenes serrados hacia la porción distal de la lámina, escariosa, con venas evidentes en cara abaxial. Florescencias breves de 1 × 0.6 cm, densas; sinflorescencias contraídas y congestas, pedúnculos y piezas florales con pelos glandulares; generalmente hipotagmas largamente desarrollados, separando por varios centímetros los paracladios basales de los apicales; brácteas florales elíptico-angostas, ca. 0.24 cm, de menor longitud que el cáliz, de ápice agudo o subobtuso; tubo corolino largo, ca. 0.62 cm, exerto ca. 0.35 cm del cáliz.

Iconografía. Briquet, 1904b: tab. 3B; Sanzin, 1919: 115, fig. 19 (flor); Chittenden, 1956: 2209; Troncoso, 1979: 243, fig. 111; Botta, 1999: 202, fig. 148.

Distribución y ecología. Verbena bonariensis var. *conglomerata* presenta una distribución más restringida que la variedad tipo, aunque es simpátrica con la misma. En Argentina se la halla únicamente en el noreste del territorio; también en los países limítrofes Uruguay, este de Paraguay y en el estado de Rio Grande do Sul, Brasil. Munir (2002) la cita para Australia, introducida desde Sudamérica de donde sería nativa. Habita en pajonales, entre pastizales y

terrenos bajos o bañados. También se halla en campos modificados con suelo arenoso pedregoso.

Observaciones. Las diferencias entre las dos variedades de *Verbena bonariensis* se hallan en las dimensiones de las flores, la arquitectura de la inflorescencia y la densidad de la pubescencia glandular, lo cual no justifican la separación de este taxón a nivel específico. La elongación del hipotagma, conjuntamente con las mayores dimensiones florales, determinan que *V. bonariensis* var. *conglomerata* sea más llamativa que la variedad tipo. La variedad *conglomerata* se reconoce por los congestos grupos de florescencias separadas por hipotagmas largos y por sus hojas generalmente de lámina sublinear a elíptico-angosta, de base auriculada. Este último carácter permite distinguir *V. bonariensis* var. *conglomerata* de *V. ovata* que presenta hojas ovadas, pero que comparte el mismo tipo de inflorescencia. Además *V. ovata* tiene pubescencia adpresa sin pelos glandulares en las piezas florales, y las brácteas florales siempre sobrepasando en longitud al cáliz. *Verbena intercedens* también se asemeja morfológicamente a la variedad *conglomerata*, distinguiéndose por la presencia de florescencias reunidas en paracladios trímeros laxos.

Briquet describió *Verbena inamoena* (Briquet, 1901a: 1058) y *V. bonariensis* var. *conglomerata* (Briquet, 1904b: 11). Según este autor *V. inamoena* sería vecina de *V. bonariensis* y se diferenciaría por poseer las hojas angostas, algo reflexas, florescencias más breves reunidas en inflorescencias de aspecto corimboso, y brácteas florales de ápice subobtuso, siendo acuminadas en *V. bonariensis*. La variedad *conglomerata* se diferenciaría de *V. bonariensis* var. *bonariensis* por el tubo corolino más largo, superando el cáliz 0.2–0.3 cm y las florescencias conglomeradas. El análisis del material tipo y de ejemplares de herbario permitió observar que no existen diferencias morfológicas entre *V. inamoena* y la var. *conglomerata*, consecuentemente *V. inamoena* se trata bajo la sinonimia de la var. *conglomerata*.

Rambo (1965) había sugerido que *Verbena inamoena* podría ser una variedad de *V. bonariensis* pero nunca publicó la combinación. Munir (2002) revalidó la variedad *conglomerata*, que había caído en la sinonimia de *V. bonariensis*, pero no la asoció con *V. inamoena*.

Material adicional examinado. ARGENTINA. **Buenos Aires:** Pdo. Campana, Campana, *Castro s.n.* (BAB 52324). **Corrientes:** Dpto. Sto. Tomé, Col. Garabí, *Krapovickas et al. 26005* (CTES, SI). **Entre Ríos:** Dpto. Concordia, Calabacilla, *Guaglianone et al. 3273* (SI); Dpto. Federación, Federación, *Pedersen 6238* (SI); Dpto. Gualeguaychú. Paranacito, *Burkart & Troncoso 27357* (SI). **Misiones:** Dpto. Capital, Posadas, *Rodriguez 5* (BA, SI). **Santa Fe:** Dpto. San Cristóbal, Capivara, *Franceschi 646* (SI). BRASIL. **Rio Grande do Sul:** San Gabriel, *Krapovickas & Cristóbal 11651* (CTES, SI). PARAGUAY. **Caaguazú:** Cnel. Oviedo, *Harley et al. 28106* (CTES, FCQ). **Guairá:** Colonia Independencia, ca. Villarica, *Rojas 4819* (SI). **San Pedro:** colonia Nva. Germania, *Rojas 1880* (SI). URUGUAY. **Paysandú:** Agronomía, *Rosengurtt B864* (SI). **Rocha:** La Coronilla, *Burkart 21554* (SI). **Salto:** Salto Grande, *Osten 5391* (CORD, SI). **San José:** Ao. Pereira, *Rosa-Mato 636* (BA).

3. Verbena ephedroides Cham., Linnaea 7: 260. 1832. TIPO: [Brasil.] "Brasilia, *Sellow*" (lectotipo, designado por O'Leary (2006: 494), K no visto; isotipos, K foto SI!, SI!).

Verbena ephedroides var. *entreriensis* Tronc., Darwiniana 21: 178. 1977 [1978]. Syn. nov. TIPO: [Argentina.] Entre Ríos: Dpto. La Paz, río Feliciano, *A. Burkart 25405* (holotipo, SI!).

Hierbas o sufrútices hasta de 2 m de altura, robustas, de tallo leñoso, cilíndrico en la base hasta ca. 35 cm antes de la primera ramificación, tetrágono hacia el ápice y en ramas superiores; costillas marcadas, lisas o ligeramente estriadas en las caras, tallos completamente glabros. *Hojas* de lámina entera, las basales de lámina elíptico-angosta a linear, de 1.5–4 cm, ápice agudo, sésiles de base cuneada, los márgenes lisos apenas dentados, ligera pubescencia estrigosa sobre vena media de cara abaxial, glabra en cara adaxial; ramas floríferas y nudos superiores de ramas con hojas escuamiformes pequeñas o ausencia de las mismas y aspecto subáfilo. *Florescencias* terminales, breves y anchas de 1 × 0.6 cm, alargándose en la fructificación, pedunculadas, agrupadas en paracladios laxos, sinflorescencia bracteosa, mayor a un orden de ramificación, las florescencias laterales superando a la principal, hipotagmas desarrollados; brácteas florales ovadas, de (0.12–) 0.17(–0.25) cm, ápice agudo, glabras, los márgenes brevemente pilosos. *Flores* sésiles, de corola color violáceo, lila o muchas veces blanco; cáliz de (0.18–) 0.22(–0.28) cm, con pubescencia adpreso-estrigosa densa, con 5 dientes triangulares breves, de ápice agudo; corola infundibuliforme, tubo corolino ca. 0.4–0.5 cm pubescencia pubérula vilosa a la altura de la garganta y en la fauce, limbo poco evidente. Clusas de 1.5–2 mm, dorso reticulado en tercio apical, el resto estriado, cara comisural verrucosa. Anatomía caulinar tipo B.

Iconografía. Troncoso, 1979: 238, fig. 108.

Distribución y ecología. Noreste de Argentina, este de Paraguay en el Dpto. Ñeembucú, y sur de Brasil hasta el estado de Paraná hacia el norte. Se la encuentra en suelos algo anegados; es poco frecuente.

Observaciones. *Verbena ephedroides* es morfológicamente afin a *V. alata*, *V. lindbergii* y *V. montevidensis*, especies con las que comparte la escasa pubescencia de la planta. *Verbena montevidensis* se distingue por su apariencia grácil y florescencias más delgadas, de 0.45 cm de ancho. *Verbena alata* y *V. lindbergii* se diferencian por la base foliar amplexicaule, y las florescencias agrupadas en torno a la florescencia principal subsésil.

Troncoso (1978) distingue *Verbena ephedroides* var. *entreriensis* por sus hojas lineares hasta escuamiformes, las brácteas florales de dimensiones mayores, entre 0.2–0.28 cm, clusas también mayores, de 2–2.5 mm. El análisis de abundante material de herbario permitió observar que existe una gradación continua en los caracteres anteriormente mencionados, encontrándose ejemplares con brácteas florales y hojas como las descriptas para la variedad *entreriensis* y con clusas del tamaño correspondiente a la variedad tipo (ej. *Nicora 6391*, SI!; *Cano 2705*, SI!). Consecuentemente, en el presente trabajo se sinonimiza la variedad *entreriensis* bajo *V. ephedroides*.

Nombre vulgar. "Yerba de Santa Ana" (De la Peña & Pensiero, 2004: 286).

Material adicional examinado. ARGENTINA. **Corrientes:** Dpto. Curuzú Cuatiá, *Cano 2705* (SI); Dpto. Itatí, Ea. Tuyutí, *Pedersen 5540* (CTES, SI); Dpto. Mercedes, río Miriñay y ruta 23, Ea. "La Chosa", *Schinini & Quarín 14458* (CTES). **Entre Ríos:** Dpto. Concepción del Uruguay, Concep. del Uruguay, *Lorentz 45* (CORD); Dpto. Concordia, Concordia, Pque. San Carlos a orillas del río Uruguay, 31°22′S; 58°00′W, *Morrone et al. 5300* (SI); Dpto. Federación, orillas de Mocoretá, *Nicora 6391* (SI). **Misiones:** Dpto. Cainguás, UNLP, ca. A. del Valle, *Biganzoli et al. 909* (SI). **Santa Fe:** Dpto. Garay, limite con Dpto. San Javier, *Ragonese 2798* (SI). BRASIL. **Paraná:** Guarapuava, *Reitz & Klein 17805* (SI). **Rio Grande do Sul:** Bom Jesus, Bdo. Velho, *Rambo 34721* (SI). **Santa Catarina:** Palhoça, morro do Cambirela 950 m s.m., *Klein & Bresolin 10033* (SI). PARAGUAY. **Ñeembucú:** Itai-pirú, *Schulz 7682* (CTES).

4. Verbena glabrata Kunth, Nov. Gen. Sp. (quarto ed.) 2: 276. 1818. TIPO: [Colombia.] "Crescit in Andibus Novo-Granatensium prope Almaguer et Pasto, altura 1160–1350 m", *s. coll. 2140* (holotipo, P no visto; isotipo, P foto SI!). Figura 4.

Verbena parvula Hayek in Engl., Bot. Jahrb. Syst. 42: 162. 1908. Syn. nov. TIPO: Bolivia. Tarija: 1903/4, *K. Fiebrig 3415* (holotipo, B†; isotipo, B foto F 17436!).

Verbena parvula Hayek var. *gigas* Moldenke, Phytologia 7: 85. 1959. Syn. nov. TIPO: Perú. Junín: Tarma, 3000–3200 m s.m., 20–22 Abr. 1929, *E. P. Killip & A. C. Smith 21925* (holotipo, NY no visto; isotipos, NY foto SI!, SI!).

Hierbas perennes, de 0.3–1 m de altura, tallo muchas veces leñoso en la base, subglabro, con pubescencia adpresa escasa sobre las aristas. *Hojas* de lámina entera, ovado-elíptica a obovada, de 2–3(–7.5) × 1–2.5 cm, ápice agudo, sésiles de base subamplexicaule, en hojas maduras base atenuada en un breve pecíolo abrazador, los márgenes serrados con dientes grandes ca. 0.5 cm hacia la porción distal de la lámina, pelos escasos, estrigosas en cara adaxial y sobre venas de cara abaxial. *Florescencias* terminales, en antesis cada florescencia posee un característico aspecto subcónico dado por el ápice agudo y la zona subapical más ancha por la presencia de flores abiertas; en la fructificación se elongan hasta 5(–8) cm; las florescencias se agrupan en paracladios trímeros, el entrenudo basal de la florescencia principal desarrollado, las laterales no superan a la principal, hipotagma desarrollado; sinflorescencia con ramificaciones de primer orden o mayor; brácteas florales ovadas de (0.2–)0.25(–0.33) cm, de ápice agudo, subglabras o con algunos pelos estrigosos en cara adaxial, los márgenes pilosos. *Flores* sésiles, corola color blanco, lila, celeste o violáceo, cáliz de (0.2–)0.23(–0.3) cm, con 5 dientes breves triangulares, pubescencia adpresa escasa, en ocasiones con pelos glandulares breves; corola infundibuliforme, tubo corolino breve de 0.35–0.5 cm, pubescente externamente y en la fauce; estambres insertos hacia la mitad del tubo corolino. Clusas de 1.8–2 cm, dorso reticulado en porción apical y márgenes, resto estriado, cara comisural verrucoso-papilosa. Anatomía caulinar tipo B.

Distribución y ecología. Desde México hasta Perú. Especie con una distribución geográfica extendida en el continente americano, pero escasamente coleccionada. Habita frecuentemente en altura, en suelos arcillosos, húmedos. Luteyn (1999) y Sklenár et al. (2005) la citan como el único taxón de *Verbena* que se halla en los páramos de Ecuador y Perú, a 3450 m s.m.

Observaciones. *Verbena glabrata* se reconoce fácilmente por el aspecto subcónico de sus florescencias. Schauer (1847) sinonimiza *V. glabrata* bajo *V. litoralis*; sin embargo esta última especie difiere porque la inflorescencia es mucho más desarrollada con florescencias cilíndricas no subcónicas, y ramificaciones de segundo o más órdenes.

Verbena glabrata var. *tenuispicata* Moldenke (1967: 283) es un sinónimo de *V. carolina* L. (serie *Verbena*).

Moldenke (1964c: 296) y López-Palacios (1977) señalan que el holotipo de *Verbena parvula* Hayek estaría depositado en el herbario de Viena (W), sin haberlo visto; Bruno Wallnöfer (comun. pers.) de herb. W no halló este material, ni tampoco se halló el mismo en el herbario de Münich (comun. pers., Alison Davies). Según la fotografía de F 17436 existía un material tipo en el herbario de Berlin (B), segur-

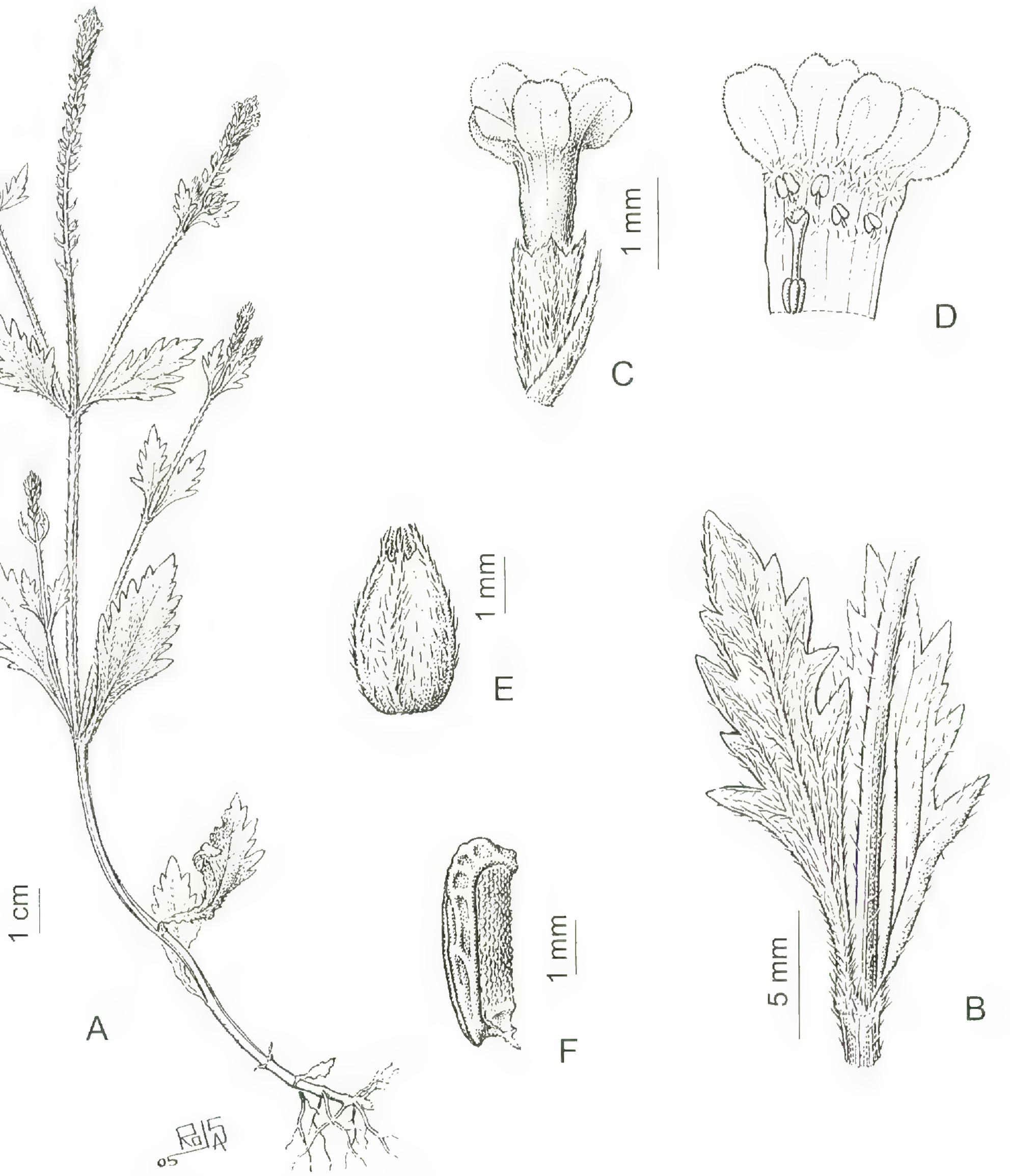


Figura 4. *Verbena glabrata*. –A. Aspecto general de la planta. —B. Detalle de nudo con dos hojas. —C. Flor. —D. Detalle corola extendida mostrando pubescencia interna y posición estambres y gineceo. —E. Cáliz fructífero. —F. Clusa. A–F: de *Sagástegui et al. 6463* (SI).

amente el holotipo actualmente destruído. Se debe lectotipificar este nombre pero hasta el presente no se han hallado duplicados de la colección original. En el protólogo Hayek describe *V. parvula* como un taxón con espigas cónicas, hojas obovadas, de pubescencia adpresa y cáliz breve; esto junto con el análisis de la foto del holotipo (B†) permite determinar que se trata de un sinónimo de *V. glabrata* Kunth.

Moldenke (1959: 85) caracteriza *Verbena parvula* var. *gigas* por los tallos erectos hasta de 90 cm de altura, sus entrenudos elongados y las hojas de lámina elíptico-oblonga, de 7.5 × 2.5 cm. El tamaño de las hojas y la elongación de los entrenudos son muy variables en *V. glabrata*, pudiéndose observar un gradiente continuo en los ejemplares analizados (ej. *Sagástegui & Aguado 2603*, SI!; *Velarde 670*, SI!). A su vez, el análisis del material tipo de la variedad *gigas* permitió determinar que no se diferencia del tipo de *V. glabrata*, consecuentemente, en el presente trabajo se incluye a esta variedad bajo la sinonimia de *V. glabrata*.

Material adicional examinado. COLOMBIA. Entre Pasto Tuquenes, 2800 m s.m., 1851–1857, *J. Triana s.n.* (P, SI). MEXICO. **Michoacán:** Morelia, Quinceo, *Arsène 3250* (BAF). **Puebla:** Moulin d'Huescotitla, *Arsène 1882* (BAF). **Vera-**

cruz: btw. Perote and Jalapa, 1.2 mi. W La Joya, *Scora 2452* (NY). PERÚ. Río Blanco, 3500–3600 m s.m., *Löthar Diers 982* (SI). **Arequipa:** Baños de Jesus, 2600 m s.m., *Sagástegui & Aguado 2603* (SI); Moquegua, Ilo, Lomas de Tacahuay, 400–700 m s.m., *FLSP 2191* (US). **Cajamarca:** Prov. Cajamarca, hacienda Polloquito, *Sanchez Vega 272* (SI); Contumazá, Cruz Gde., 3000 m s.m., *Sagástegui et al. 6463* (SI). **Junín:** Tarma, Palea, 2600–2800 m s.m., *Velarde 670* (SI). **La Libertad:** Prov. Otuzco, Los Tres Shulgones, *Leiva & Leiva 76* (SI).

5. Verbena hispida Ruiz & Pav., Fl. Peruv. 1: 22. 1798. TIPO: Perú. 1778–88, *H. Ruiz & J. A. Pavón 1/34* (lectotipo, designado por Munir (2002: 47), MA no visto; isotipos, BM no visto, G no visto, MA foto F 29686!, MA[2] foto SI!, P foto SI!, SI!).

Hierbas perennes o sufrútices, de 30–50(–80) cm de altura, con ramas decumbentes en la base y algo aplicadas al suelo y luego erectas y erguidas, tallos tetrágonos ramosos, toda la planta con pubescencia variable, desde esparcidamente pubérula adpresa hasta híspida densa. *Hojas* de lámina entera, de (3–)4–6(–10) × 1.5–2.5 cm, oblongas, elípticas u ovado-angostas, a veces algo trífidas en la base, ápice agudo u obtuso, sésiles, base subamplexicaule a cuneado-atenuada, márgenes lisos hacia la base, irregularmente inciso-serrados en la mitad distal de la lámina, dientes de ápice agudo-acuminado; cara adaxial estrigosa y abaxial densamente híspida con pelos glandulares en ambas superficies o pubescencia estrigosa en ambas caras sin pelos glandulares; venas prominentes en el envés. *Florescencias* terminales, densas, cilíndricas de 4–8(–15) × (0.45–)0.8(–1) cm, dos brácteas frondosas opuestas basales a las florescencias más proximales; agrupadas en paracladios laxos de tres ó más florescencias, los laterales superando la florescencia principal, pedúnculos breves o desarrollados, hipotagmas desarrollados, sinflorescencia frondosa con ramificaciones de primer orden; bráctea floral elíptica u ovado-angosta, de (0.23–)0.3(–0.45) cm, ápice agudo-acuminado y márgenes pilosos, algo mayores o iguales en longitud que el cáliz. *Flores* sésiles, corola color azul, violáceo, lila o blanco, cáliz de 0.2–0.35 cm, con 5 dientes triangulares de ápice agudo, pubescencia variable desde híspida con abundantes pelos glandulares largos a subglabra con pelos adpresos sin pelos glandulares; corola infundibuliforme, tubo corolino ca. 0.4–0.5 cm, pubescente en la mitad superior, viloso en la fauce; estambres insertos debajo de la mitad del tubo corolino. Clusas de 1.5–2 mm, dorso reticulado y estriado, cara comisural verrucosa o muricada. Anatomía caulinar tipo B.

Observaciones. Muchas veces las flores presentan notoria coloración violácea en la bráctea floral, o en el margen de la misma, o en los dientes del cáliz, dando a la florescencia una coloración general violácea.

Número cromosómico. $n = 7$ (Noack, 1937); $2n = 14$ (Junell, 1934; Dermen, 1936).

CLAVE DE LAS VARIEDADES DE *VERBENA HISPIDA*

5a. Tallos, hojas y piezas florales híspidos con pubescencia de pelos patentes, con pelos glandulares abundantes y largos; florescencias anchas de (0.6–)0.8(–1) cm de ancho . . . . . 5a. var. *hispida*

5b. Tallos, hojas y piezas florales pubérulos con pubescencia de pelos adpresos, sin pelos glandulares o raramente presentes, cuando presentes escasos y breves; florescencias angostas de 0.45–0.67 cm de ancho . . . . . 5b. var. *obovata*

5a. **Verbena hispida** var. **hispida** Ruiz & Pav.

Verbena intermedia var. *lanuginosa* Moldenke, Phytologia 3: 118. 1949. Syn. nov. TIPO: Argentina. Mendoza: Dpto. Tunuyán, San Pablo, La Pampa, 1935, *R. A. Ruíz Leal 2981* (holotipo, NY no visto; isotipos, MERL foto SI!, NY foto SI!, SI!).

Verbena parvula f. *albiflora* Moldenke, Phytologia 61: 444. 1987. Syn. nov. TIPO: Bolivia. Tarija: Cercado, área Ceramitar, 4 Dic. 1985, *E. Bastián 36* (holotipo, TEX no visto; isotipos, TEX foto SI!; SI!).

Plantas con tallos de pubescencia híspida muy evidente, áspera al tacto. Hojas con la cara adaxial estrigosa y abaxial densamente híspida, con pelos glandulares en ambas superficies, venación evidente. Florescencias de (0.6–)0.8(–1) cm de ancho, híspidas glanduloso-pubescentes, brevemente pedunculadas; brácteas florales elípticas, de 0.3–0.45 cm, híspidas con abundantes pelos glandulares largos; cáliz de 0.2–0.35 cm, híspido con pelos glandulares largos. Clusas de dorso reticulado en el tercio superior, resto estriado, cara comisural muricada.

Iconografía. Ruiz y Pavón, 1798: 1, t. 34, fig. a; Sanzin, 1919: 116, fig. 20 (flor); Botta y Cabrera, 1993: 92, fig. 41; Munir, 2002: 49, fig. 3.

Distribución y ecología. Norte y noroeste de Argentina, Paraguay, norte y centro de Chile, Bolivia, sur y centro de Perú. Munir (2002) la cita para el sudeste de Australia, donde sería un taxón introducido desde Sudamérica. Crece en bosques marginales, matorrales, rastrojos, es muy común a lo largo de rutas. Habita preferencialmente terrenos bajos, de suelo franco-arcilloso, húmedo.

Observaciones. Las dos variedades se distinguen principalmente por el tipo de pubescencia, la variedad *obovata* posee pelos adpresos, sin pelos glandulares y la variedad tipo se caracteriza por tener

pelos patentes, híspidos y con abundantes pelos glandulares.

Verbena cuneifolia Hort. ex Walp. es un nombre inválido ya que carece de una diagnosis y designación de tipo, Walpers (1845: 22) lo cita bajo la sinonimia de *V. hispida*. *Verbena glandulosa* (Moris, 1830), también es un nombre inválido, cuyo ejemplar tipo pudo provenir de un ejemplar cultivado en los Jardines Botánicos de Turin a partir de semillas mandadas desde Chile por Bertero, pero ahora imposible de localizar. Schauer (1847) la cita bajo la sinonimiza de *V. hispida*.

Tipificaciones y nomenclatura. El tipo de *Verbena intermedia* var. *lanuginosa* que se encuentra depositado en el herbario NY posee inflorescencia de *V. hispida*, pero toda la planta parecería deformada, sobre todo las hojas, muy gruesas de nervios marcados. Las flores son semejantes a las presentes en *V. hispida*, y la diferencia radica en que poseen hojas revolutas y pubescencia vilosa. Es probable que este ejemplar corresponda a una planta de *V. hispida* deformada por algún patógeno.

Material adicional examinado. ARGENTINA. **Catamarca:** Dpto. Del Alto, Balicozna, *Venturi 7229* (SI). **Chaco:** Dpto. Tapenagá, Enrique Urién, Campo Bonazzola, *Rodrigo 2432* (LP, SI). **Córdoba:** Dpto. Minas, La Bismutina, *Castellanos s.n.* (BA 51170). **Formosa:** Dpto. Patiño, ca. Palo Santo, *Petetin 1672* (BAB); Dpto. Pilcomayo, Cn. Palma Sola, *Schinini et al. 31322* (CTES); Punta Porá, Tres Lagunas, *Rojas 8893* (BAF, SI). **Jujuy:** Dpto. El Carmen, Las Lajitas, *Krapovickas et al. 26557* (CTES, LP, SI). **La Rioja:** Dpto. Gral. Belgrano, entre Montenegro y Pozo de la Vaca, *Riedel 110* (CTES, SI). **Mendoza:** Dpto. Las Heras, San Ignacio, *Ruíz Leal 9337* (SI). **Misiones:** Dpto. Gral. M. Belgrano, Urugua-í, *Múlgura et al. 2774* (CTES, SI). **Salta:** Dpto. Capital, Cpo. Militar Gral. Belgrano, *Novara 7313* (SI). **San Juan:** Dpto. Sarmiento, río Bachongo, *Guaglianone et al. 1435* (SI). **San Luis:** Bajo de Velis, *Kurtz 8595* (LP). **Santa Fe:** Dpto. San Cristóbal, ca. Ceres, *Burkart et al. 30597* (SI). **Santiago del Estero:** Dpto. Robles, Beltrán, *Maldonado 496* (LP, SI). **Tucumán:** Dpto. Capital, Barrancas Coloradas, *Venturi 825* (BA, SI); Dpto. Leales, Los Luamanos, *Venturi 525* (SI). BOLIVIA. **Cochabamba:** Prov. Cercado, valle Cochabamba, *Steinbach 84* (CTES). **Chuquisaca:** Prov. Luis Calvo, El Salvador, *Saravia Toledo 2879* (CTES). **La Paz:** Prov. Murillo, ca. Calacoto, 3300 m s.m., *M. Nee 30410* (MO); Prov. Sud Yungas, Iquico, 3440 m s.m., *B. Vuilleumier 333* (MO); Los Andes, 6.6 km NW Batallas, rd. to Titicaca, *J. C. Solomon 11467* p.p. (MO); Prov. Murillo, Alto Segúencoma, *Solomon 15028* (CTES); orillas Titicaca, Tiquina, *Cocucci et al. 3287* (CTES). **Santa Cruz:** Prov. Cordillera Alto Parapetí, *Michel et al. 306* (SI). **Tarija:** Prov. Eustaquio Mendez, *Beck et al. 22029* (SI). CHILE. **I Región:** Arica, Valle Azapa, *Escobar X-200* (SI). **V Región:** Viña del Mar, *Hicken 116* (SI). PARAGUAY. **Alto Paraguay:** Pto. Casado, obraje Mosquito, *Rojas 1890* (AS, SI). PERÚ. Licuari, 3550 m s.m., *Hicken s.n.* (SI 10519); Yucay, *Soukup 259* (BA). **Cajamarca:** Contumazá, Teterillas-Marín, Guzmango, *Sagástegui 10377* (NY). **Lima:** Lima, Sta. Eulalia, *Velarde 1664* (SI). **Puno:** Chunto, *Anibal Vera s.n.* (SI). **Tacna:** Tarata, Ticaco, 3600–4000 m s.m., *Cano 8335* (US).

5b. Verbena hispida var. **obovata** (Moldenke) N. O'Leary, comb. nov. Basónimo: *Verbena parvula* Hayek var. *obovata* Moldenke, Phytologia 36: 52. 1977. TIPO: Ecuador. Pichincha: Quito, 2 Jun. 1977, *S. López-Palacios 4250* (holotipo, TEX no visto; isotipos, SI!, TEX foto SI!). Figura 3E–G.

Verbena bangiana Moldenke, Phytologia 3: 63. 1949. Syn. nov. TIPO: Bolivia. Sorata, Feb. 1886, *H. Rusby 911* (holotipo, NY no visto; isotipos, NY foto SI!, SI!, US!).

Plantas con tallos de pubescencia esparcidamente pubérula adpresa. Hojas de pubescencia estrigosa en ambas caras, con venas prominentes en la cara abaxial y venas secundarias subparalelas, emergiendo a la altura del pecíolo marginado, conniventes hacia el ápice. Florescencias de 0.45–0.67 cm de ancho, pedúnculos desarrollados de pubescencia pubérula o estrigosa; brácteas florales ovado-angostas, de (0.23–)0.28–0.32(–0.4) cm, glabras a subglabras en la cara abaxial, marginalmente ciliadas, sin pelos glandulares o apenas algunos; cáliz de (0.2–)0.25(–0.3) cm, pubescencia adpresa sobre costillas. Clusas de dorso reticulado en la mitad apical, el resto estriado, cara comisural verrucosa.

Iconografía. López-Palacios, 1977: 572, fig. 134 (sub nom. *V. parvula* Hayek).

Distribución y ecología. La distribución de la variedad *obovata* es más restingida que la de la variedad tipo, encontrándose en el noroeste de Argentina y en Bolivia, en los departamentos de Tarija, La Paz y Cochabamba. López-Palacios (1977) la cita para Ecuador y Venezuela. Se la encuentra en acequias al borde de caminos y sobre suelo arcilloso húmedo.

Observaciones. *Verbena parvula* var. *obovata* fue considerado un taxón válido por Moldenke (1977a) por tener hojas elípticas a obovadas, para diferenciarla de *V. parvula* var. *parvula*. Sin embargo el análisis de ambos materiales tipo permitió determinar que *V. parvula* var. *parvula* y la var. *obovata* son dos taxones diferentes, siendo el primero sinónimo de *V. glabrata* y el segundo una variedad de *V. hispida*. *Verbena parvula* var. *obovata* comparte con *V. hispida* las florescencias densas, agrupadas en sinflorescencias frondosas y la hojas ovadas de margenes lisos hacia la base, sin embargo la variedad *obovata* difiere en la pubescencia y las dimensiones florales. Estos dos caracteres no se consideran suficientemente importantes en *Verbena*

como para sustentar una especie diferente, por lo que este taxón se trata aquí como una variedad de *V. hispida*.

Verbena hispida var. *obovata* es afin a *V. litoralis* y a *V. glabrata*, en cuanto a la morfología de la inflorescencia, sin embargo ésta última se distingue por la forma subcónica de las floresencias y *V. litoralis* se diferencia por la pubescencia menor, casi subglabra en los tallos y la sinflorescencia bracteosa (vs. frondosa) de menor grosor, llegando excepcionalmente a 0.6 cm de ancho.

La descripción de *Verbena parvula* de López-Palacios (1977), conjuntamente con la lámina de referencia (fig. 134), se corresponden con *V. hispida* variedad *obovata* por poseer las florescencias cilíndricas, ya que *V. parvula* variedad *parvula* (que es un sinónimo de *V. glabrata* Kunth) posee las florescencias subcónicas.

Moldenke (1949a: 63) al describir *Verbena bangiana* señala que se asemeja a *V. litoralis* en cuanto al hábito, mientras que la inflorescencia se asemeja a *V. hispida*. El estudio del material tipo de *V. bangiana* permitió determinar que no existen diferencias con la var. *obovata*. Moldenke (1962: 201), menciona la posibilidad de que *V. bangiana* sea un híbrido entre *V. hispida* y *V. litoralis*, porque compartiría caracteres morfológicos con ambos taxones, y menciona un híbrido artificial entre ambas especies producido por Dermen (1936). Sin embargo no se puede afirmar que sea un híbrido por las semejanzas morfológicas sin tener otros datos que lo confirmen, como análisis cromosómicos.

Material adicional examinado. ARGENTINA. **Jujuy:** Dpto. Ledesma, Pque. Nac. Calilegua, Aguas Blancas, 2600 m s.m., *Ramadori 198* (SI). **Salta:** Dpto. La Caldera, Yacones, 1500 m s.m., *Novara 1905* (SI). **Tucumán:** Dpto. Trancas, La Angostura, poco antes de Tafí del Valle, *J. Caro 5000* (BAF). BOLIVIA. **Cochabamba:** Morochata, 18 Nov. 1903, *Dereims s.n.* (P, SI); s. loc., 18 Dic. 1921, 1800 m s.m., *Steinbach 6009* (SI); Morochata, 3550 m s.m., *Beck 7402* (SI). **La Paz:** Prov. Sud Yungas, Lambate, río Chiquilaque Muriyaqui, *Solomon 17545* (CTES); Prov. Sud Yungas, 3100 m s.m., *Beck 3905* (SI); Nor Yungas, río Unduavi, 1920 m s.m., *Steibel 903* (SI); Prov. Murillo, Murillo, 3300 m s.m., *Solomon 12759A* (SI); Prov. Ingaví, 3850 m s.m., *Solomon 13254* (SI); Prov. Ingaví, ca. Hacienda Lacaya, 20 km NW Tambillo ca. Titicaca, *Gentry et al. 44367* (MO); Prov. B. Saavedra, Kanton Amarete, 3750 m s.m., *Krach 7793* (SI); Prov. Los Andes, 6.6 km NW Batallas, rd. to Titicaca, *Solomon 11467* p.p. (MO). **Tarija:** Prov. Cercado, 2650 m s.m., *Bastián 492* (SI); De Tarija a Iscayachi, 2000–3000 m s.m., *Kiesling et al. 3797* (SI).

6. Verbena intercedens Briq., Bull. Herb. Boissier, sér. 2(4): 1057. 1904. TIPO: Paraguay. "Caraguatay, in campis humidis", 1898–1899, *E. Hassler 3324* (lectotipo, designado por O'Leary (2006: 496), G 8696/270 no visto; isotipos, G 8696/270 foto SI!, G 8696/271 no visto, K foto SI!, SI!). Figura 5.

Verbena bonariensis var. *hispida* Moldenke, Phytologia 33: 374. 1976. Syn. nov. TIPO: Brasil. Rio Grande do Sul: Villa Germanica, Mar. 1906, *A. Bornmüller 647* (holotipo, M no visto; isotipo, M foto SI!).

Hierbas anuales o perennes, erectas, robustas, hasta de 1.5 m de altura, tallos y ramas tetrágonos con pubescencia escabrosa en las aristas, sin pelos glandulares. *Hojas* de lámina entera, de 4–6 × 1–3 cm, oblongas u ovadas, ápice agudo u obtuso, sésiles de base subauriculada a auriculada, los márgenes uniformemente serrados, algo coriáceas, rígidas y rugosas, con pelos escabrosos en ambas caras, envés notariamente reticulado-venoso, con abundante venación secundaria reticulada entre las venas principales; disminuyendo en tamaño hacia el ápice de las ramas, siendo éstas casi áfilas. *Florescencias* terminales, cilíndricas densas, de 2–5 × 0.7–0.8 cm en antesis, reunidas en paracladios trímeros laxos, no aglomerados en torno a la florescencia principal, superándola, pedúnculos de entre 0.5–1 cm, sinflorescencia bracteosa de más de un orden de ramificación; brácteas florales ovado-angostas, de. 0.4–0.55 cm, igual o mayores en longitud que el cáliz, ápice agudo, híspidas con pelos glandulares. *Flores* sésiles, corola color azul, purpúreo, violáceo o lila; cáliz de (0.25–)0.31(–0.35) cm, híspido, con 5 dientes triangulares de ápice agudo, ambas piezas florales color verde amarillento pálido, muchas veces violáceos, dando a la florescencia esta coloración; corola infundibuliforme, tubo corolino largo de (0.7–)0.9(–1.2) cm, lóbulos bastante evidentes, pubescente externamente, fauce apenas pubérula. Clusas ca. 2 mm, dorso reticulado en el tercio superior, resto estriado, cara comisural papilosa. Anatomía caulinar tipo B.

Distribución y ecología. Argentina en la provincia de Corrientes, este de Paraguay y sur de Brasil en el estado de Rio Grande do Sul. Crece en pantanos, campos bajos o en lomas y pastizales.

Observaciones. *Verbena intercedens* es morfológicamente similar a *V. intermedia* y *V. rigida*, compartiendo el tipo de pubescencia híspido-glandular de las piezas florales y la morfología de la inflorescencia, formada por paracladios trímeros laxos no aglomerados en torno a la florescencia principal, con las florescencias laterales superándola. *Verbena intercedens* se diferencia de ambas especies principalmente por la ausencia de pelos glandulares en los tallos, por su mayor porte y por la división del márgen de las hojas.

Briquet (1904a: 1057) fundó *Verbena intercedens* como especie híbrida producto de *V. bonariensis* L. ×

V. ovata Cham., basándose en que comparte similitudes morfológicas con ambos taxones. Esto resulta insuficiente para definir un híbrido, por lo tanto se la considera como especie válida hasta que no se realicen estudios más detallados. *Verbena bonariensis* posee las hojas comparativamente menos rugosas y rígidas y las flores más pequeñas que las de *V. intercedens*. *Verbena ovata* difiere porque ésta no posee pelos glandulares en las piezas florales y posee generalmente las hojas ovadas.

El ejemplar tipo de *Verbena bonariensis* var. *hispida* no difiere morfológicamente de *V. intercedens* por lo cual en el presente tratamiento se incluye bajo la sinonimia de la misma.

Material adicional examinado. ARGENTINA. **Corrientes:** Dpto. Sto. Tomé, 1 Dic. 1970, *Krapovickas et al. 16795* (CTES, SI). BRASIL. **Rio Grande do Sul:** Alegrete, 5 Dic. 1978, *Krapovickas & Cristóbal 34164* (CTES). PARAGUAY. **Guairá:** Cnia. Independencia, 12 Dic. 1989, *Mereles 3763* (CTES, FCQ); Cnia. Independencia, Mar. 1924, *T. Rojas 4819a* (AS, MO, SI). **Paraguarí:** Tebicuary 1941, *Rojas 9278* (SI); Cerros de Tobay, año 1900, *Hassler 6149* (paratipo fotografía F 17422, GH, K, NY, P, SI).

7. Verbena intermedia Gillies & Hook. ex Hook., Bot. Misc. 1: 166. 1829. TIPO: [Argentina.] Pampas y Buenos Ayres, *Gillies s.n.* (holotipo, K no visto; isotipo, K foto SI!).

Verbena tenuis Steud., Nomencl. Bot. ed. 2(2): 751. 1841. Nombre reemplazado: *Verbena gracilis* Cham., Linnaea 7: 261. 1832, nom. illeg., non *Verbena gracilis* Desf., Cat. Pl. Horti. Paris ed. 3: 393. 1829. *Verbena bonariensis* f. *gracilis* (Cham.) Voss, Vilm. Blumengärtn. ed. 3(1): 826. 1894. *Verbena chamissonis* Walp., Repert. Bot. Syst. 4: 18. 1845, nom. illeg. superfl. TIPO: [Brasil.] Bras. merid., *F. Sellow* (lectotipo, designado por O'Leary (2006: 496), K no visto; isotipos, BR foto SI!; K foto SI!).

Verbena bonariensis var. *longibracteata* Kuntze, Revis. Gen. Pl. 3(2): 255. 1898. TIPO: Argentina. Buenos Aires: Ventana, *R. Hauthal 649* (holotipo, NY no visto; isotipos, NY foto SI!, SI!).

Verbena tandilensis Speg., Contr. Fl. Tandil, 41. 1901. TIPO: Argentina. [Buenos Aires:] Tandil, Ene. 1901, *C. L. Spegazzini 2409* (lectotipo, designado por O'Leary (2006: 498), LP!).

Verbena carollata Briq., Annuaire Conserv. Jard. Bot. Genève 10: 101. 1907. TIPO: Argentina. Mendoza: Sa. Pintada, 1897, *E. Wilczek 56* (holotipo, G no visto; isotipos, F foto SI!, G foto SI!, SI!).

Verbena litoralis var. *melanopotamica* Hauman, Anales Mus. Nac. Hist. Nat. Buenos Aires 24: 413. 1913. Syn. nov. TIPO: Argentina. Río Negro: *L. Hauman-Merck 345* (holotipo, BA!; isotipo, SI!).

Verbena intermedia f. *albiflora* Moldenke, Phytologia 3: 176. 1949. Syn. nov. TIPO: Argentina. Entre Ríos: Gualeguaychú, 5 Ene. 1932, *A. Burkart 4179* (holotipo, MVM no visto; isotipo, SI!).

Hierbas anuales o perennes, erectas, de 0.5–1 m de altura, tallos tetrágonos longitudinalmente estriados, entrenudos alargándose hacia el ápice, estrigosos con algunos pelos glandulares, áspero-escabrosos en las aristas. *Hojas* de lámina entera, oblongo-elípticas-angostas, de (1–)5–6(–8) × (0.2–)1(–5) cm, ápice agudo, sésiles de base subamplexicaule a subauriculada, subrígidas, con pubescencia de pelos breves, callosos en cara abaxial, venas principales prominentes en el envés, venación secundaria poco evidente; las hojas basales provistas de 2–3 pares de dientes opuestos o casi alternos en la mitad distal de la lámina, las hojas superiores sublineares con los márgenes lisos, generalmente dispuestas de forma erecta, algo adpresas al tallo. *Florescencias* terminales, cilíndricas densas, breves de 2–4 cm alargadas hasta 8 cm en la fructificación; reunidas en paracladios trímeros, los laterales superando a la florescencia principal, las florescencias basales largamente pedunculadas, las superiores brevemente, sinflorescencia bracteosa de más de un orden de ramificación; brácteas florales ovado-angostas, acuminadas, de 0.3–0.4 cm, los márgenes ciliados, generalmente de igual o menor longitud que el cáliz, en ocasiones mayores. *Flores* sésiles, corola color azul, purpúreo, rosa o blanca; cáliz de (0.25–)0.3–0.5 cm, con 5 dientes triangulares de ápice agudo; pubescencia de brácteas, cáliz y pedúnculos con pelos híspidos y pelos glandulares; corola infundibuliforme, tubo corolino de (0.4–)0.5–0.7(–0.9) cm, generalmente el doble en longitud que el cáliz, viloso en la garganta, fauce subglabra apenas pubérula, limbo desarrollado, estambres en la garganta corolina. Clusas de 1.8–2 mm, dorso reticulado en el tercio superior, resto estriado, cara comisural verrucosa. Anatomía caulinar tipo B.

Número cromosómico. $2n = 56$ (Schnack & Covas, 1946, 1947) y $5x = 35$ (Schnack & Covas, 1947).

Iconografía. Troncoso, 1939: 481, fig. 1; Cabrera, 1953: 396, fig. 148a–e; Marzocca, 1963: 323, fig. 61B; Troncoso, 1965: 130, fig. 45e–g; Troncoso, 1979: 237, fig. 107; Lombardo, 1983: 54, lam. 19(2).

Distribución y ecología. Centro, norte y noreste de Argentina, este de Paraguay, Uruguay y sur de Brasil en los estados de Rio Grande do Sul y Santa Catarina. Se la puede encontrar en campos bajos, inundables, pajonales; también en terrenos pedregosos y secos, o en suelos arenosos pero fértiles. Muchas veces al borde del camino, en las vías del tren o en suelos modificados. Constituye una maleza en montes frutales del noreste de Entre Ríos, donde en ocasiones llega a tener tendencia invasora (Marzocca, 1963: 321).

Figura 5. *Verbena intercedens.* —A. Aspecto general de la planta con inflorescencia. —B. Detalle de la pubescencia de la cara adaxial de la hoja. —C. Flor. A–C: de *Rojas 9278* (SI).

Observaciones. *Verbena intermedia* es una especie morfológicamente variable, las flores poseen la corola desde 0.4 cm a casi 1 cm, el ancho de las florescencias puede oscilar entre 0.45 cm y 1 cm.

Verbena intermedia es fácilmente diferenciable por sus hojas subrígidas de margen liso o con dientes marcados hacia la porción distal de la lámina, y sus florescencias con pubescencia hirsuto-glandular reunidas en paracladios trímeros, con flores llamativas por sus largos tubos corolinos. *Verbena intermedia* se asemeja a *V. rigida* por el tipo de pubescencia y la disposición de las florescencias, apartándose esta última por tener flores de corola hipocraterimorfa con tubo corolar comparativamente mayor de (0.5–)0.65–0.9(–1.1) cm y hojas de márgenes uniformemente inciso-serrados con dientes agudos de senos cóncavos. *Verbena intermedia* también puede confundirse con *V. litoralis*, distinguiéndose esta última por las menores dimensiones florales, la pubescencia estrigosa y el tipo de inflorescencia formada por paracladios multímeros.

Hauman (1913: 413) al describir la variedad *melanopotamica* señala que posee brácteas florales más largas y flores más conspicuas que la variedad tipo. Al analizar el material se observó que las medidas de los caracteres anteriormente mencionados no difieren de *Verbena intermedia*, por lo cual este taxón se considera bajo la sinonimia de *V. intermedia*.

Briquet (1907: 101) describe la especie *Verbena carollata* diferenciándola de *V. litoralis* por su corola más exerta, su cáliz más largo y las florescencias casi el doble de anchas. El ejemplar tipo de este taxón proviene de Argentina, de la provincia de Mendoza; se trata de un ejemplar de *V. intermedia* evidentemente malformado, por algún fitopatógeno. Consecuentemente, en el presente trabajo se incluye a *V. carollata* bajo la sinonimia de *V. intermedia*, de acuerdo con Moldenke (1964a: 377).

Moldenke (1949c: 176) diferencia *Verbena intermedia* forma *albiflora* por el color de la corola. Sin embargo, este carácter no justifica el establecimiento de un taxón infraespecífico basado en él y en este trabajo se trata bajo la sinonimia de *V. intermedia*.

Se observó en ejemplares provenientes de la Isla Martín García, Argentina, una variación morfológica de *Verbena intermedia* que no merece valor taxonómico. Estos ejemplares poseen hojas trífidas en la base de la lámina, marcadamente dentadas, piezas florales muy hispídulas con pelos glandulares, brácteas apenas superando el cáliz y corola breve hasta de 0.6 cm (ej. *Hurrel & Belgrano 2788*, SI!; *Hurrel et al. 2222*, SI!).

Nombre vulgar. "Siempre Viva del Campo" (De la Peña & Pensiero, 2004: 286).

Material adicional examinado. ARGENTINA. **Buenos Aires:** Pdo. Cnel. Suárez, Cura Malal, *Hicken 19* (BAF); Pdo. Pellegrini, Pellegrini, *Cabrera 6925* (LP, SI); Pdo. Tigre, Isla Martín García, *Hurrel & Belgrano 2788* (SI); *Hurrel et al. 2222* (SI). **Chaco:** Dpto. 1° de Mayo, Cnia. Benítez, *Schulz 8794* (BAB). **Córdoba:** Dpto. Río Cuarto, Ao. La Barranquita, *Krapovickas & Seijó 47516* (CTES). **Corrientes:** Dpto. Concepción, Ea. Sta. Rosalía, *Pedersen 12005* (CTES, SI); Dpto. Mburrucuyá, Ea. Sta. Teresa, *Pedersen 4660* (CTES, SI). **Entre Ríos:** Dpto. Gualeguaychú, Urdinarrain, *Burkart 26036* (SI); Dpto. La Paz, Ea. Sta. Elena, *Pensiero 1745* (SI); Dpto. Paraná, Hernandarias, *Burkart et al. 25410* (SI). **Formosa:** Dpto. Patiño, ca. San Carlo, *Fortunato et al. 2408(1/2)* (BAB). **La Pampa:** Dpto. Loventué, Loventué, *Fortuna 76* (BA). **La Rioja:** Dpto. Gral. Ocampo, Sa. de los Llanos, *Schinini et al. 6248* (CTES). **Mendoza:** Dpto. San Rafael, Los Reyunos, *Huajardo 4840* (SI); Dpto. Tupungato, Tupungato, *Ruiz Leal 1498* (SI). **Misiones:** Dpto. Apóstoles, Concepción de la Sierra, *Krapovickas et al. 15319* (CTES). **Salta:** Dpto. Guachipas, Cuesta del Lajar, *Novara & Bruno 9412* (SI). **San Juan:** Dpto. Jáchal, Qda. de las Vacas, 2450 m s.m., *Kiesling et al. 9465* (SI). **San Luis:** Dpto. Pedernera, Est. Lavaise, Las Alamos, *Rosa 1340* (CTES). **Santa Fe:** Dpto. San Lorenzo, Carcarañá, *Bermdt 5160* (CORD); Dpto. 9 de Julio, Ao. Golondrina, *Fortunato 185* (BAB). **Santiago del Estero:** Dpto. Ojo de Agua, Las Lomitas, *Pérez Moreau 3860* (BAB). BRASIL. **Rio Grande do Sul:** ca. Rosario do Sul, *Arbo & Schinini 2454* (CTES). **Santa Catarina:** São Joaquin, *Smith & Reitz 14330* (SI). PARAGUAY. **Misiones:** Santiago, Ea. La Soledad, *Pedersen 7680* (SI). URUGUAY. s. loc., 1918, *Felippone 2057* (SI). **Canelones:** Pedrera, *Rosengurtt B1915* (SI). **Cerro Largo:** Río Negro y Ao. Palleros, *Rosengurtt 1002* (SI). **Florida:** A Mansavillagra, *Rosengurtt 765* (SI). **Colonia:** Cnia. Suiza, *De la Rua 77* (SI). **Montevideo:** Peñarol, *Rosengurtt 535* (SI). **Río Negro:** El Greco, *Solís Neffa et al. 87* (CTES). **Tacuarembó:** s. loc., 8 Feb. 1981, *Cabrera & Zuloaga 32420* (SI).

8. Verbena lindbergii Moldenke, Phytologia 2: 322. 1947. TIPO: Brasil. Minas Gerais: Caldas, Ribeiro dos Buggris, Nov. 1854, *S. O. Lindberg 136* (holotipo, BR no visto; isotipos, BR foto SI!, NY no visto, SI!). Figura 6.

Verbena malmei Moldenke, Phytologia 2: 475. 1948. Syn. nov. TIPO: Brasil. Rio Grande do Sul: Villa Rica, 22 Ene. 1902, *G. O. A. Malme 1141* (holotipo, S no visto; isotipos, NY foto SI!, S foto SI!, SI!).

Verbena reitzii Moldenke, Phytologia 3: 119. 1949. Syn. nov. TIPO: Brasil. Santa Catarina: Campo dos Padres, 16 Dic. 1948, *P. R. Reitz 2366* (holotipo, NY no visto; isotipos, NY foto SI!, SI!).

Verbena alata f. *alba* Moldenke, Phytologia 3: 426. 1951. Syn. nov. TIPO: Brasil. Minas Gerais: Serra de Caldas, *A. F. Regnell I326* (holotipo, NY no visto; isotipos, NY foto SI!, SI!).

Hierbas perennes, erectas, hasta de 2 m de altura, tallo y ramas tetrágonas, glabras. *Hojas* de lámina entera, las basales de 2(–4) × 0.5(–1) cm, elíptico-angostas, o sublineares, ápice agudo, sésiles de base subamplexicaule, los márgenes lisos o apenas dentados con 1–3 dientes muy breves a cada lado en

porción distal de la lámina, subglabras; entrenudos breves, los nudos superiores con hojas reducidas escuamiformes. *Florescencias* terminales, numerosas, de 1 × 0.50(–0.55) cm, breves, subcapitadas, densas, multifloras, reunidas en paracladios laxos o a veces algo congestos debido a los pedúnculos breves, a veces el entrenudo basal de la florescencia principal poco desarrollado y entonces aspecto subcorimbiforme, hipotagma desarrollado, florescencias laterales superando o no a la principal, sinflorescencias bracteosas, ramificaciones de segundo o mayor orden; brácteas florales ovadas, de 0.15–0.2 cm, acuminadas, los márgenes pilosos. *Flores* sésiles, de corola color celeste, lila o blanco; cáliz siempre de mayor longitud que brácteas, ca. 0.26 cm, con 5 dientes triangulares breves, de ápice agudo, glabro o con pelos esparcidamente adpreso-estrigosos; corola infundibuliforme, tubo corolino ca. 0.45–0.5 cm con pubescencia ligeramente pubérula en la parte externa de la garganta, con pelos moniliformes en la fauce, limbo desarrollado de 0.3–0.4 cm. Clusas de 1.5–2 mm, dorso reticulado en el tercio superior, el resto estriado, cara comisural papilosa. Anatomía caulinar tipo B.

Distribución y ecología. Sur de Brasil en los estados de Rio Grande do Sul, Santa Catarina, São Paulo y Paraná. Crece a la vera de arroyos en terreno húmedo, se la encuentra en bosquecillo ribereño.

Observaciones. *Verbena lindbergii* es morfológicamente afin a *V. alata*, *V. ephedroides* y *V. montevidensis* en cuanto a la escasa pubescencia (véase diferencias con *V. alata* y *V. ephedroides* bajo sus respectivas observaciones). *Verbena montevidensis* se diferencia de *V. lindbergii* porque posee la base foliar cuneada, porte grácil y florescencias delgadas y breves de 0.4–1(–2) × 0.45 cm.

Moldenke (1948b: 475) caracteriza *Verbena malmei* por tener principalmente brácteas florales pequeñas, ca. 0.1 cm, y hojas pequeñas, ca. 1 cm. Sin embargo, Moldenke basó la descripción de su especie únicamente en el ejemplar tipo; este ejemplar está incompleto ya que carece de la porción basal de la planta, motivo por el cual Moldenke describe la planta como de aspecto áfilo con hojas pequeñas. Por otro lado, un año antes, Moldenke (1947) describe *V. lindbergii* y la define como planta de hojas pequeñas hasta de 4 cm y brácteas florales ca. 2.5 cm. El análisis del material de herbario permitió observar que existe una gradación contínua en los caracteres anteriormente mencionados; generalmente la porción apical de la planta presenta los caracteres que distinguen a *V. malmei* y en la porción más basal los que diferencian a *V. lindbergii* (ej. *Dombrowski 7735*, SI!; *Koezicki 83*, SI!; *Reitz 2344*, SI!). Consecuentemente, en el presente trabajo ambos taxones son considerados coespecíficos.

El análisis del ejemplar tipo de *Verbena alata* f. *alba* de Moldenke (1951: 426) permitió determinar que no posee los nudos contraídos característicos de *V. alata* y en cambio presenta hojas en los nudos superiores, como ocurre en *V. lindbergii*. Por lo tanto este taxón se trata bajo la sinonimia de *V. lindbergii*.

Verbena reitzii Moldenke (1949b: 119) agrupa aquellos ejemplares con florescencias reunidas en paracladios trímeros, de forma fastigiada, con hojas grandes. A partir del análisis del material de herbario (ej. *Krapovickas & Cristóbal 37802*, SI!) y del material tipo de *V. reitzii* se observa que los caracteres que la definen entran dentro de la variación morfológica de *V. lindbergii*. Consecuentemente se incluye *V. reitzii* bajo la sinonimia de *V. lindbergii*.

Material adicional examinado. BRASIL. **Paraná:** Barigui, Curitiba, *Hatschbach 25327* (CTES, SI); Capão da Imbuía, *Dombrowski 2272* (SI); Piraguará, S. Roque, *Saito 436* (CTES); Piraguará, *Dombrowski 7735* (SI); Rio Cavernoso, *Zanardini s.n.* (SI); San José dos Pinhais, col. Roseira, *Koezicki 83* (SI); San Sepé, *Hagelund 12174* (SI); Tijucas do Sul, Curitiba, *Krapovickas & Cristóbal 33615* (CTES). **Rio Grande do Sul:** Banhado seco, *Linderman s.n.* (CTES, ICN 9446); S. Francisco Paula, *Burkart 25164* (SI); Bom Jesus, *Hagelund 8875* (SI). **Santa Catarina:** São Joaquin, orilla de arroyo, *Krapovickas & Cristóbal 37802* (CTES); fazenda dos Carneiros, Caçador, *Klein 3528* (SI); Campo dos Padres, Bom Retiro, *Reitz 2344* (SI); Serra do Matador, rio do Sul, *Reitz & Klein 6840* (SI); Campo dos Padres, *Smith & Reitz 10303* (SI). **São Paulo:** Mogydas Cruzas, *Glaziou 17716* (P, SI).

9. Verbena litoralis Kunth, Nov. Gen. Sp. (quarto ed.) 2: 276, t. 137. 1817 [1818]. *Verbena bonariensis* var. *litoralis* (Kunth) Gillies & Hook. ex Hook., Bot. Misc. 1: 166. 1829. TIPO: [Perú.] Trujillo, *A. Bonpland s.n.* (lectotipo, designado por Macbride, (1960: 624), P no visto; isotipo, SI!).

Hierbas perennes, de 0.8–3 m de altura, tallos ramificados, tetrágonos, ramas erectas, ligeramente pubescentes con pelos breves estrigosos cuando jóvenes, subglabros a la madurez, ligeramente escabrosos en las aristas. *Hojas* de lámina entera, forma variable elíptico-ovadas, oblongas, obovadas, de 5–12 × 1–3 cm, ápice obtuso o agudo, cuneadas gradualmente hacia un breve pecíolo o subsésiles de base ligeramente subamplexicaule, excepcionalmente auriculada, los márgenes irregularmente dentado-serrados, a veces bi-dentados, o algo lobados a casi lisos o escasamente serrados, principalmente en hojas jóvenes, ligeramente pubescentes en cara abaxial, con pelos escabroso-estrigosos sobre las venas. *Florescencias* terminales, cilíndricas de 2–5(–18 fructificación) × 0.4–0.55 cm, la principal más brevemente pedunculada que las laterales, los paracladios laterales

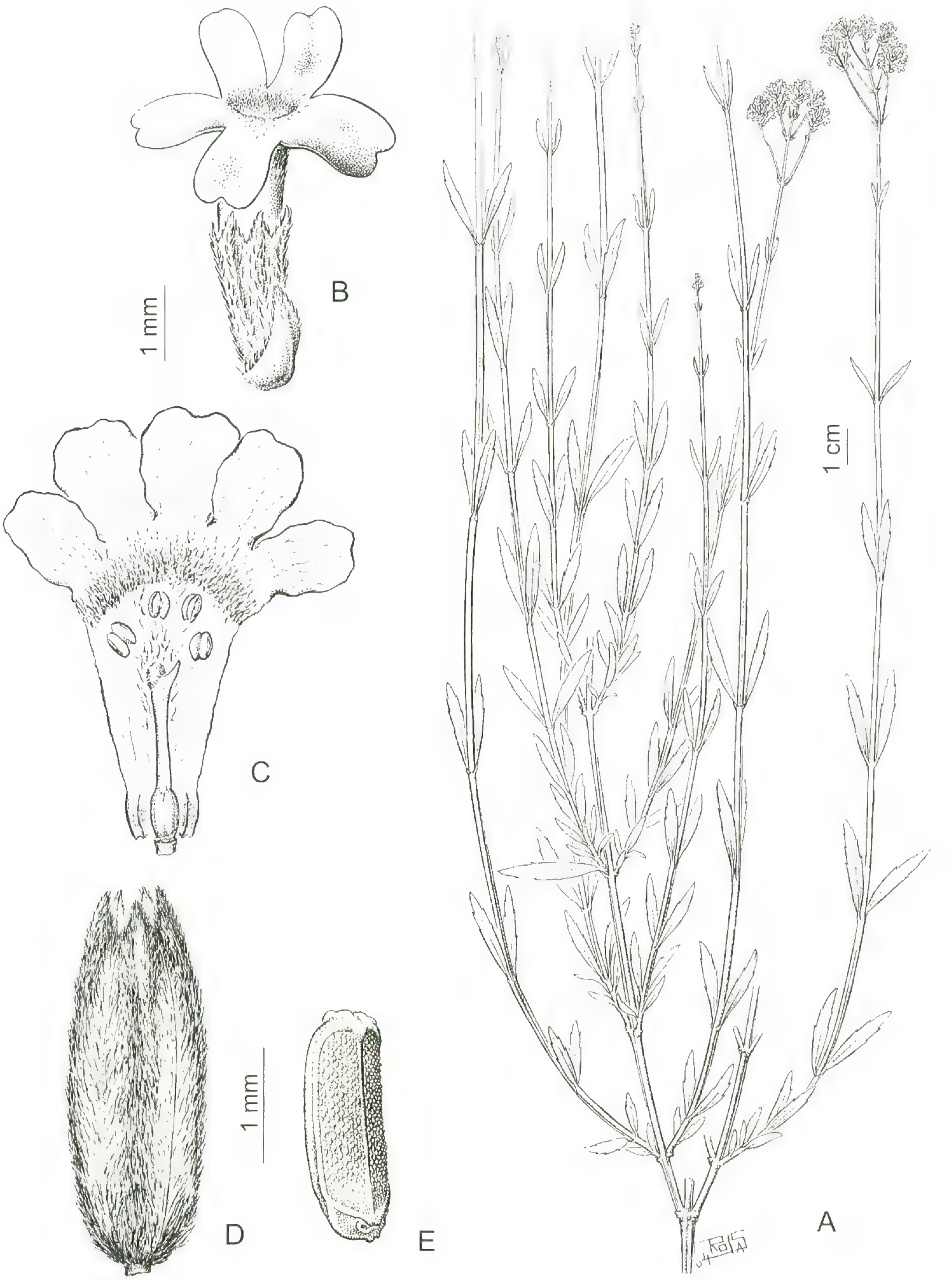


Figura 6. *Verbena lindbergii*. —A. Aspecto general de la planta. —B. Flor. —C. Corola extendida mostrando pubescencia interna y posición de estambres y gineceo. —D. Cáliz fructífero. —E. Clusa. A: de *Klein 3528* (SI); B, E: de *Smith & Reitz 10303* (SI); C: de *Reitz 2344* (SI); D: de *Dombrowski 7735* (SI).

superando a la florescencia principal, generalmente de alto orden de ramificación dando aspecto paniculiforme, sinflorescencia bracteosa; pedúnculos y raquis delgados, no glandulosos, algo pubérulos a glabros en la madurez, tetrágonos, de 3–12 cm: brácteas florales ovadas, acuminadas, de márgenes ciliados, glabras adaxialmente, de (0.15–)0.2–0.35 (–0.4) cm, longitud similar que el cáliz o algo menor, ocasionalmente mayor que el cáliz. *Flores* sésiles, corola color violáceo, lila o blanco; cáliz de 0.2–0.3 (–0.4) cm, estrigoso-piloso, con 5 dientes triangulares breves de ápice agudo; corola infundibuliforme, tubo corolino apenas superando el cáliz, de 0.3–0.5 cm long., viloso a apenas pubérulo en la garganta, fauce subglabra a pubérula, limbo reducido, poco evidente; estambres insertos debajo de la garganta corolina. Clusas de 1.5 mm, dorso reticulado en el tercio superior, el resto estriado, cara comisural muricada a verrucosa. Anatomía caulinar tipo B.

Número cromosómico. $2n = 28$ (Dermen, 1936; Noack, 1937).

Distribución y ecología. *Verbena litoralis* es un taxón ampliamente distribuido, existiendo diferentes formas según el lugar donde habite. Nativa de Sudamérica, en la actualidad se la encuentra en Norte y Centroamérica, Sudáfrica, Australia e islas de Pacífico (Munir, 2002: 69). Posee afinidad por ambientes disturbados.

Observaciones. *Verbena litoralis* es la especie de *Verbena* más común en el continente sudamericano, es un taxón morfológicamente muy variable a lo largo de su rango de distribución, consecuentemente numerosas categorías infraespecíficas han sido reconocidas y descriptas, generando una gran cantidad de nombres. Los caracteres que presentan alto polimorfismo son principalmente la ramificación de las inflorescencias, la mayor o menor densidad de flores en las florescencias, la longitud y ancho de las florescencias y el tipo de hojas: la morfología, tamaño, pilosidad, márgenes y textura.

Schauer (1847) y Briquet (1904c) distinguen dos variedades de *Verbena litoralis* basadas en la elongación relativa de las florescencias. Posteriormente, Perry (1933) reconoce ambas variedades a nivel específico. Yeo (1990) también las trata como especies, pero sugiere la posibilidad de que fueran subespecies de *V. litoralis*. Munir (2002) reconoce nuevamente bajo *V. litoralis* dos variedades. En este trabajo se sigue el criterio de Munir (2002) reconociendo dos variedades de *V. litoralis*, la variedad tipo y la variedad *brevibracteata*.

A *Verbena litoralis* se le atribuyen propiedades medicinales hepáticas, antigangrenosas y se menciona como remedio contra resfriados del pecho. Un reciente estudio sobre la histomorfología y análisis fitoquímico de *V. litoralis* (Souza et al., 2005) revela que sería un buen insumo farmacéutico por la ausencia de cristales de oxalato de calcio.

Nombre vulgar. "Verbena amarga", "verbena del litoral", "verbena encañutada", "yerba del acero", "yerbón" (De la Peña & Pensiero, 2004: 286).

Tipificaciones y nomenclatura. Munir (2002) aclara que Kunth no especificó cual de las tres localidades correspondía al tipo de *Verbena litoralis*, tratándose entonces de 3 sintipos, por lo cual en 1960 cuando Macbride (1960: 624) menciona "Trujillo, Bonpland, Type" se trata de una lectotipificación (Art. 7.11, Greuter et al., 2000). Igualmente Munir (2002) señala que no se han hallado los ejemplares de las otras dos localidades.

CLAVE DE LAS VARIEDADES DE *VERBENA LITORALIS*

9a. Hojas de base atenuada cuneadas hasta ligeramente subpecioladas, éste siempre menos a 0.5 mm, las basales hasta de 5 × 2.5 cm; florescencias con pubescencia pubérula escasa sin pelos glandulares, flores distantes en la parte basal del raquis, brácteas florales ovadas, flores de tubo corolino menor de 0.4 cm . . . . . 9a. var. *litoralis*

9b. Hojas de base subamplexicaule, excepcionalmete auriculada, las basales pueden tener hasta 10 cm; florescencias con pubescencia pilosa, algo estrigosa, a veces portando algunos pelos glandulares en piezas florales, flores densamente imbricadas a lo largo del raquis, brácteas florales ovado-angostas, flores ca. 0.45 cm . . . 9b. var. *brevibracteata*

9a. **Verbena litoralis** var. **litoralis** Kunth

Verbena caracasana Kunth, Nov. Gen. Sp. (quarto ed.) 2: 275. 1818. *Verbena litoralis* var. *caracasana* (Kunth) Briq., Annuaire Conserv. Jard. Bot. Genève 7-8: 292. 1904. nom. illeg. TIPO: [Venezuela.] Caracas, *F. W. Humboldt & A. Bonpland 658* (lectotipo, designado por O'Leary (2006: 494), P no visto; isotipos, P foto SI!. SI!).

Verbena affinis M. Martens & Galeotti, Bull. Acad. Roy. Sci. Bruxelles 11(2): 322. 1844. TIPO: México. Michoacán: Morelia, 1956 m s.m., 1840, *H. G. Galeotti 781* (holotipo, BR no visto; isotipo, BR foto SI!).

Verbena litoralis var. *leptostachya* Schauer, Prodr. 11: 542. 1847. TIPO: México. Ad Jalapam et Papantlam, Ago. 1828, *C. J. W. Schiede 1168* (lectotipo, designado por Munir (2002: 65), HAL 076812 no visto).

Verbena nudiflora Nutt. ex Turcz., Bull. Soc. Imp. Naturalistes Moscou 36(2): 195. 1863. TIPO: Sandwich Isles. Ouan, *T. Nuttall 1835* (holotipo, BM no visto; isotipo, BM foto SI!).

Verbena litoralis f. *angustifolia* Chodat, Bull. Herb. Boissier, sér. 2(9): 818. 1902. Syn. nov. TIPO: Paraguay.

Tucangua, Feb. 1897, *E. Hassler 3853* (holotipo, BM no visto; isotipos, G no visto, NY foto SI!, SI!).

Verbena approximata Briq., Annuaire Conserv. Jard. Bot. Genève 7-8: 292. 1904. TIPO: Paraguay. Grand Chaco, en face de l'Assomption, 1875, *B. Balansa 1027c* (holotipo, P no visto; isotipos, G no visto, P foto SI!, SI!).

Verbena cordobensis Briq., Annuaire Conserv. Jard. Bot. Genève 10: 100. 1907. Syn. nov. TIPO: Argentina. Córdoba: Ea. Germánica, *P. G. Lorentz 131* (holotipo, M no visto; isotipo, M foto F 20316!).

Verbena sphaerocarpa L. M. Perry, Ann. Missouri Bot. Gard. 20: 256. 1933. Syn. nov. TIPO: México. Colima: Socorro Island. Mar.–Jun. 1897, *A. W. Anthony 380* (holotipo, MO no visto; isotipos, G no visto, GH foto SI!, MO foto SI!, UC foto SI!, US foto SI!).

Verbena litoralis var. *albiflora* Moldenke, Phytologia 1: 432. 1940. *Verbena litoralis* f. *albiflora* (Moldenke) Moldenke, Phytologia 44: 329. 1979. TIPO: México. Michoacán: Coalcoman, 1939, *G. B. Hinton 13965* (holotipo, LA no visto; isotipo, US foto SI!).

Verbena integrifolia Sessé & Moç. f. *albiflora* Moldenke, Phytologia 16: 95. 1968. Syn. nov. TIPO: México. Michoacán: Zamora, 20 Jun. 1967, *H. Moldenke 1750* (holotipo, TEX no visto; isotipos, TEX foto SI!, SI!).

Verbena litoralis var. *portoricensis* Moldenke, Phytologia 50: 310. 1982. Syn. nov. TIPO: Puerto Rico. Panoramic Highway south of Cayey, 640 m, 14 Mar. 1979, *A. Liogier & Martorell 28417* (holotipo, NY no visto; isotipo, NY foto SI!).

Planta hasta de 2.5–3 m de altura, tallos y ramas erectos, marcadamente tetrágonos, glabros o algo escabrosos en las aristas. *Hojas* elípticas, de 3–10 × 2.5 cm, de base atenuado-cuneada a subpeciolada, éste menor a 0.5 mm, los márgenes uniformes o irregularmente serrado-dentados o lisos; cartáceas. *Florescencias* de 2.5–10 × 0.4 cm en antesis, luego elongándose y con flores más laxamente dispuestas principalmente en la base del raquis, reunidas en sinflorescencias de alto grado de ramificación; brácteas florales ovadas, ca. 0.25 cm, de similar longitud que el cáliz, o algo mayores, pubescencia de pelos estrigosos no glandulares; tubo corolino ca. 0.3–0.4 cm, apenas exerto del cáliz.

Iconografía. Kunth, 1818: t. 137; Sanzin, 1919: 114, fig. 18; Troncoso y Burkart, 1946: 211, fig. 2b (corte transversal tallo); Marzocca, 1963: 323, fig. 611; Moldenke, 1971: 507, fig. 133; Moldenke, 1973: 46, fig. 1; Troncoso, 1979: 242, fig. 110; Lombardo, 1983: 54, lam. 20(1); Yeo, 1990: 116, fig. 4; Botta, 1999: 202, fig 146; Munir, 2002: 68, fig. 6.

Distribución y ecología. *Verbena litoralis* es nativa de Sudamérica, actualmente distribuida por América cálida y templada. En Argentina se la encuentra desde la latitud 40 en el sur hacia el norte, siendo muy abundante en la zona mesopotámica y el noroeste (Tucumán, Jujuy, Salta); también se la encuentra en Chile, Bolivia, este de Paraguay, Uruguay, sur de Brasil hasta el estado de Minas Gerais y en Perú, llegando su distribución hasta Colombia, Venezuela, Honduras y México. Munir (2002) la cita para la costa este de Australia, como introducida desde Sudamérica. Crece cerca del agua, en campos húmedos y bajos; prefiere terreno inculto, de tierra calcárea, arenosa o suelos fértiles. También se la ha encontrado en matorrales, pastizales, prados o áreas abiertas y disturbadas del bosque, orillas de montes, borde del camino y precipicios rocosos.

Observaciones. *Verbena litoralis* es morfológicamente afin a *V. montevidensis* distinguiéndose esta última por su menor altura, tallos glabros, florescencias breves ca. 4 cm nunca mayores de 8 cm, y menores dimensiones florales con brácteas florales de (0.08–)0.15–0.18(–0.27) cm.

En Chile (*Cabrera 31920*, SI!; *Hicken 113*, SI!) y Bolivia (*Buchtien s.n.*, SI 3402!) se han coleccionado ejemplares que poseen hojas trilobadas en la base y los márgenes serrados a biserrados, lo cual se correspondería con el material tipo de la variedad *portoricensis* de Moldenke (1982: 310). Munir (2002) sinonimiza bajo *Verbena litoralis* esta variedad, basándose en la descripción sin haber visto el material tipo. El análisis del material tipo y de ejemplares de herbario (*Buchtien 197*, SI!; *Gay 794*, SI!) con hojas típicas de *V. litoralis* y hojas levemente trilobadas en el mismo ejemplar permitió determinar que no existen caracteres suficientes para considerar la existencia de un taxón subespecífico, aceptándose la sinonimia propuesta por Munir (2002).

Se han encontrado ejemplares con florescencias laxas, gráciles, más delgadas y largas, y flores más espaciadas en la parte basal de la florescencia (ej. *Cabrera 8198*, SI!; *Humbert et al. 27159*, SI!). Estos ejemplares se corresponderían con lo que Kunth (1818) llamó *Verbena caracasana* o la variedad *leptostachyae* de Schauer, pero no merecen valor taxonómico, ya que los caracteres que los distinguen se encuentran dentro de la variación específica de *V. litoralis*.

Perry (1933) señala que *Verbena sphaerocarpa* es similar a *V. litoralis* en cuanto al hábito, pero se diferenciaría porque las florescencias de *V. sphaerocarpa* son más breves y densas, las flores más pequeñas y el esquizocarpo aún no dividido es casi tan ancho como largo. El análisis del ejemplar tipo evidencia que estas diferencias no permiten separarla de *V. litoralis* y que se trataría de un sinónimo de la misma.

Martens y Galeotti (1844) mencionan bajo el protólogo de *Verbena affinis* que es "affinis *V.*

bonariensis", pero dista mucho de ser así ya que la inflorescencia es morfológicamente similar a la presente en *V. litoralis*.

Moldenke (1942a: 428) coloca a *Verbena approximata* Briq. como sinónimo de *V. brasiliensis* Vell. sin haber visto el tipo del primer taxón. Troncoso (1979) en cambio, sinonimiza *V. approximata* bajo *V. litoralis*. El análisis del material tipo de *V. approximata* permitió determinar que los caracteres morfológicos se corresponden con los de *V. litoralis*, consecuentemente se considera a *V. approximata* bajo la sinonimia de *V. litoralis*.

El tipo de *Verbena litoralis* f. *angustifolia* Chodat presenta hojas angostas que entran dentro de la variación intraespecífica de este taxón, por lo cual esta forma se incluye en la sinonimia.

Tipificaciones y nomenclatura. En 1818 Kunth funda *Verbena litoralis* y *V. caracasana* Kunth. En 1847 Schauer reconoce dos variedades de *V. litoralis*, (1) *V. litoralis* var. *pycnostachya* Schauer, con *V. litoralis* Kunth como sinónimo de la misma, por lo cual esta variedad no está válidamente publicada (Art. 26.2, Greuter et al., 2000) ya que contiene al tipo de la especie y debería ser variedad *litoralis*; (2) *V. litoralis* var. *leptostachya* Schauer. Posteriormente, Briquet (1904c: 292) combina *V. caracasana* de Kunth como variedad de *V. litoralis*: *V. litoralis* var. *caracasana* (Kunth) Briq., y coloca *V. litoralis* var. *leptostachya* Schauer como sinónimo, por lo tanto la variedad *caracasana* de Briquet es ilegítima por ser un nombre supérfluo (Art. 52, Greuter et al., 2000) ya que posee el mismo tipo que la variedad *leptostachya* de Schauer, no pudiendo tener otro nombre.

El ejemplar tipo de *Verbena cordobensis* fue coleccionado por Lorentz en 1874 y en ese momento determinado por Grisebach como *V. ephedroides*. Briquet (1907) se basa en ese ejemplar como tipo de su nueva especie *V. cordobensis*. Posteriormente, Moldenke (1942a), sinonimiza *V. cordobensis* bajo *V. brasiliensis*. El material tipo de *V. cordobensis* depositado en el Herb. M no presenta diferencias morfológicas con *V. litoralis*, por lo cual se trata *V. cordobensis* bajo la sinonimia de *V. litoralis*.

Material adicional examinado. ARGENTINA. **Buenos Aires:** Pdo. San Isidro, San Isidro, reserva Ribera Norte, *O'Leary 528* (SI). **Catamarca:** Dpto. Ambato, Ao. Piedra Aspera, *Saravia Toledo et al. 13130* (CTES). **Chaco:** Dpto. 1° de Mayo, Cnia. Benítez, *Schulz 8755* (BAB). **Córdoba:** Dpto. San Justo, Arroyito, *Krapovickas et al. 18526* (CTES, SI). **Corrientes:** Dpto. Ituzaingó, Isla Apipé Grande, *Krapovickas et al. 23980* (CTES, SI). **Entre Ríos:** Dpto. Federación, Ea. Buena Esperanza, *Pedersen 8127* (SI). **Formosa:** Dpto. Patiño, ca. Cnel. Fontana, *Petetin et al. 1796* (BAB). **Jujuy:** Dpto. El Carmen, El Carmen, *Cabrera & Kiesling 20382* (LP, SI). **La Pampa:** Dpto. Realicó, Ing. Luigi, *O'Leary 501* (SI). **La Rioja:** Dpto. Castro Barros, Qda. de los Molinos, *Di Iorio 07* (SI). **Mendoza:** Dpto. Rivadavia, Luján de Cuyo, ruta 49, *Naranjo et al. 873* (SI). **Misiones:** Dpto. San Pedro, Pque. Prov. Moconá, *Zuloaga et al. 5452* (CTES, SI). **Neuquén:** Dpto. Chos Malal, Huengaucó, *Correa et al. 10498* (BAB). **Río Negro:** Dpto. Conesa, Gral. Conesa, *Sánchez et al. 169* (BAB). **Salta:** Dpto. Anta, El Quebrachal, *Luna 167* (BA); Dpto. Capital, Cerro San Bernardo, *Cabrera 8198* (SI). **San Juan:** Dpto. Valle Fértil, San Agustín de Valle Fértil, *Kiesling et al. 4948* (SI). **San Luis:** Dpto. Capital, San Luis, entre Co. Piedra y San Francisco, 1925, *Delétang & Guiñazú s.n.* (BAB). **Santa Fe:** Dpto. Gral Obligado, Lanteri, *Maldonado & Bruzzone 1723* (SI). **Santiago del Estero:** Dpto. Copo, ca. Monte Quemado, *Fortunato et al. 1574* (BAB, SI). **Tucumán:** Dpto. Capital, Villa Luján, *Venturi 50* (SI). BOLIVIA. Cotoña, am Ibimani, 2450 m s.m., *Buchtien 197* (BAF, SI 3401). **La Paz:** Prov. Nor Yungas, Chuspipata, *Solomon 8601* (CTES). **Santa Cruz:** s. loc., 18 Jun. 1916, *Steinbach 2368* (SI); region andina, 1911, *Buchtien s.n.* (SI); Prov. Florida, ca. Achiras, *Nee M. & D. Atha 50079* (CTES). **Tarija:** Rincón de la Victoria, *Krapovickas et al. 18874* (CTES, LP, SI). BRASIL. **Minas Gerais:** Pr. Viçosa, *Emmerich 89* (SI). **Paraná:** Barigui, *Dombrowski 1852* (SI). **Rio de Janeiro:** Rio de Janeiro, Jardim Primavera, *Pabst 5425* (SI). **Rio Grande do Sul:** Porto Alegre, Jardín Botánico, *Bueno 2707* (CTES). **Santa Catarina:** Itapiranga, *Smith & Klein 14102* (SI). CHILE. s. loc., 1839, *Gay 794* (SI); s. loc., 20 Mar. 1921, *Hicken 113* (SI); **IV Región:** Prov. Aconcagua, Tilama, *Fraga 18* (SI). **V Región:** Valparaíso, *Buchtien* 976 (BAF, SI); Isla de Pascua, *Cabrera 31920* (SI); cerro Cruz. Limache, *Garaventa 1661* (BAA, CTES). **VI Region:** Prov. Colchagua, Nancagua, *Ricardi 10241* (SI). COLOMBIA. **Antioquia:** Mpio. de Sabanalarga, via entre El Oro y El Placer, *Callejas et al. 2288* (SI). **Cundinimarca:** route de Villavicencio, Guayabetal, *Humbert et al. 27159* (SI); Quetame, *Zuloaga 3968* (SI). HONDURAS. **El Paraíso:** co. Montserrat, *Nayda Castro T. 125* (MO). MÉXICO. **Chiapas:** Chiapas, 20 mi. SW Ocosingo Hwy. 199, 1400 m s.m., *T. B. Croat 40401* (MO); Santiago Tuxtla, *Martínez 1471* (LP). **Hidalgo:** Santiago, Municipio San Bartolo, Tutotepec, *Gimate 625* (CTES). **Oaxaca:** río Molino, 4 km SW San Miguel Suchistepec, *J. Rzedowski 20988* (NY). **Sonora:** Hermosilla, *F. Malebig 204* (US). **Veracruz:** Gorge at Puente Acabaloya, 1 km SE Xico Viejo, *M. Nee & K. Taylor 26318* (NY). PARAGUAY. **Alto Paraná:** ca. Hernandarias, *Hahn et al. 924* (PY; SI). **Amambay:** camino a Co. Corá, *Morrone & Pensiero 411* (SI). **Caaguazú:** Ao. Guaranungua, *Zardini & Velásquez 24656* (AS, MO, SI). **Caazapá:** Tovai, Pacuri, *Basualdo 211-4* (FCQ, MO, SI). **Canindeyú:** Cnia. Mboi Yagua Pindoty, *Basualdo 6028* (FCQ). **Central:** Trinidad, Asunción, *Pérez 243* (AS, MO, SI). **Concepción:** A° Tagatijami, *Gonzalez & Mora 941* (FCQ). **Cordillera:** Tovatí, *Zardini & Velásquez 26656* (FCQ). **Guairá:** Cord. de Ibytyruzú, 13 Mar. 1989, *Zardini & Velásquez 11568* (FCQ, MO, SI). **Misiones:** Santiago, *Pedersen 8625* (SI). **Ñeembucú:** s. loc., 2 Mayo 1949, *Ramirez 341* (SI). **Paraguarí:** Pque. Nac. Ybycui, *Zardini & Velásquez 10177* (MO, PY, SI). **San Pedro:** Est. Sta. María, *Wolf et al. 55* (SI). PERÚ. **La Libertad:** Prov. Trujillo El Cortijo, *Sagastegui 7192* (SI). PUERTO RICO. **Aibonito:** Aibonito, rd. 173, *Woodbury & García Llano s.n.* (MO 4231380). **Cayey:** route 7722, 2 km N of route 1, *Miller et al. 6450* (MO). **Coamo:** *Woodbury & García Llano s.n.* (MO 4231372). **Villalba:** Toro Negro forest, Rd. 149 ca. Maravilla, *P. Acevedo Rdgz. 2980* (MO). URUGUAY. **Salto:** Ao. Arerungua, Picada de Pereyra, *Rosengurtt B1016* (SI). **Paysandú:** Escuela Agronómica, 1937, *Rosengurtt B865* (SI). VENEZUELA. Distrito Federal, Los Chorros, *Burkart*

16016 (SI). **Cojedes:** San Carlos, *Burkart 16170* (SI). **Mérida:** Acarigua, Camburito, Edo. Portuguesa, *Burkart 17069* (SI). **Sucre:** Cuamana, *Funck 54* (SI). **Trujillo:** Cacute, *Burkart 16815* (SI).

MASCARENE ISLANDS. Mauritius, *D. Lorence 178* (MO).

SUDÁFRICA. Komgha, Transkei, *Bayliss 3045* (MO); Colchester, near Port Elizabeth, *Bayliss 6985* (MO); Eastern cape, near Ida, *Bayliss 7937* (MO).

9b. Verbena litoralis var. **brevibracteata** (Kuntze) N. O'Leary, comb. nov. Basónimo: *Verbena bonariensis* var. *brevibracteata* Kuntze, Revis. Gen. Pl. 3(2): 254. 1898. TIPO: Argentina. Tucumán: Sa. de la Cuesta del Garrabal, 30 Ene. 1874, *P. G. Lorentz & Hieronymus 853* (lectotipo, designado por O'Leary (2006: 494), NY no visto; isotipos, CORD!, NY foto SI!, SI!). Figura 7A, B.

Verbena quadrangularis Vell., Fl. Flumin. 16. 1825; Icon. 1: tab. 39. 1827. TIPO: Vellozo, 1827: Icon. 1: tab. 39 (lectotipo, designado por Verdcourt (1992: 10)).

Verbena brasiliensis Vell., Fl. Flumin. 17. 1825; Icon. 1: tab. 10. 1827. *Verbena litoralis* var. *brasiliensis* (Vell.) Munir, J. Adelaide Bot. Gard. 20(1): 71. 2002. TIPO: Vellozo, 1827: Icon. 1: tab. 40 (lectotipo, designado por Verdcourt (1992: 9)).

Verbena paucifolia Turcz., Bull. Soc. Nat. Mosc. 36(2): 196. 1863, nom. illeg., non *Verbena paucifolia* M. Martens & Galeotti, Bull. Acad. Roy. Sci. Bruxelles 11(2): 324. 1844. Syn. nov. TIPO: Estados Unidos de América. Texas: *M. Botteri s.n.* (holotipo, P no visto; isotipo, P foto SI!).

Verbena hanseni Greene, Pittonia 3: 308. 1898. TIPO: [Estados Unidos de América.] Sequoia Gigantea Region. Amador Co., Clinton, 1896, *Geo. Hansen 2025* (holotipo, NDG 043279 no visto; isotipos, K no visto, NDG foto SI!, SI!).

Verbena intermedia f. *glabrescens* Hauman, Anales Mus. Nac. Hist. Nat. Buenos Aires 24: 413. 1913. Syn. nov. TIPO: Argentina. Río Negro: *L. L. Hauman-Merck 344* (holotipo, BA!).

Verbena brasiliensis var. *subglabrata* Moldenke, Phytologia 3: 278. 1950. Syn. nov. TIPO: Chile. San Fernando, Colchagua, Feb. 1930, *E. Barros 8050* (holotipo, NY no visto; isotipo, SI!).

Verbena chacensis Moldenke, Phytologia 5: 228. 1955. TIPO: Paraguay. Gran Chaco, Loma Clavel, Nov. 1903, *E. Hassler 2459* (holotipo, UC no visto; isotipos, P foto SI!, SI!, TEX foto SI!, UC foto SI!).

Verbena litoralis var. *congesta* Moldenke, Phytologia 20: 80. 1970. *Verbena litoralis* f. *congesta* (Moldenke) Moldenke, Phytologia 44: 329. 1979. Syn. nov. TIPO: México. Sinaloa: Sa. Surutto, Badiraguato, Los Ornos, 1969, *D. E. Breedlove & F. S. Kawahara 16735* (holotipo, TEX no visto; isotipos, SI!, TEX foto SI!).

Planta de 2–2.5 m de altura, robusta muchas veces sufrutescente o leñosa en la base, tallos de aristas agudas. *Hojas* elípticas, de 8–10 cm, sésiles de base subcuneada, subamplexicaule, a veces ligeramente auriculada; los márgenes espaciadamente serrados o desde la mitad distal de la lámina, con dientes cóncavos, acuminados; cartáceas a coriáceas. *Florescencias* de 2.5–8 × 0.55 cm, densas, flores congestas a lo largo del raquis, no espaciadas en la parte basal del raquis cuando fructifica; las laterales más largamente pedunculadas, la florescencia principal sésil o subsésil; brácteas florales ovado-angostas, ca. 0.35 cm, de igual o mayor longitud que el cáliz, pubescencia estrigosa, pilosa, con o sin pelos glandulares pero escasos y siempre cortos; tubo corolino ca. 0.45 cm.

Número cromosómico. $n = 14$, $2n = 28$ (Lewis & Oliver, 1961).

Iconografía. Gleason, 1952: 128; Yeo, 1990: 110, fig. 3.

Distribución y ecología. Habita en el centro y norte de Argentina, Chile desde la X región en el sur hasta la I región; Bolivia, este de Paraguay, Perú, sur de Brasil. Naturalizada en Estados Unidos de América, se la ha encontrado en Texas, Carolina del Sur y Massachusetts. En Europa se encuentra recientemente naturalizada en Cataluña, España (Verloove, 2003) y en Italia (Soldano, 2000); Munir (2002) la cita para la costa este de Australia, introducida desde Sudamérica. Habita sobre suelo seco-pedregoso, salino o también arenoso, en áreas disturbadas.

Observaciones. Kuntze (1894: 255) caracteriza *Verbena bonariensis* var. *brevibracteata* por las brácteas florales breves; el análisis del material tipo permitió determinar que este taxón es muy similar a *V. litoralis* pero difiere en cuanto a la base de las hojas y la disposición de las florescencias, entre otros caracteres. Moldenke (1964a: 376) trata *V. bonariensis* var. *brevibracteata* Kuntze bajo la sinonimia de *V. intermedia*, pero esta variedad de Kuntze no posee pelos glandulares, lo cual es característico en *V. intermedia*.

Moldenke (1970) al describir *Verbena litoralis* var. *congesta* la distingue de la variedad tipo por poseer las florescencias abreviadas y congestas. La variedad *congesta* posee semejanzas morfológicas con *V. bonariensis*, por presentar ambos taxones las florescencias densas y las flores dispuestas de manera congesta a lo largo del raquis. Sin embargo, *V. bonariensis* se distingue por las hojas de base auriculada, pubescencia escabrosa a híspida y florescencias más breves hasta de 6 cm. Por lo tanto la variedad *congesta* se trata bajo la sinonimia de *V. litoralis* var. *brevibracteata*.

Hauman (1913) y Moldenke (1950) describen a *Verbena intermedia* f. *glabrescens* y *V. brasiliensis*

var. *subglabrata*, respectivamente, y distinguen ambos taxones de la variedad tipo por la escasa pubescencia. El ejemplar tipo de la forma *glabrescens* se asemeja morfológicamente a la variedad *brevibracteata* de Kuntze, pero con menor pubescencia, lo mismo ocurre con el tipo de la variedad *subglabrata*. El análisis de materiales de herbario (ej. *Ruiz Huidobro 1473*, SI!; *Sayago 989*, SI!; *Pedersen 9867*, SI!), permitió observar una gradación continua de la densidad de la pubescencia; por lo tanto en el presente trabajo ambos taxones se tratan bajo la sinonimia de la variedad *brevibracteata*.

Michael (1997) sugiere que el taxón *Verbena brasiliensis* Vell. se trataría de un híbrido dado que observó ejemplares con malformaciones en la corola y falta de semillas, y se basa en los dibujos y el protólogo de la *Flora Fluminensis* donde se señala que presenta flores aberrantes y dos semillas en vez de cuatro "duo semina habet oblonga" (Vellozo, 1825). Por lo tanto propone que todos aquellos taxones que se asemejen a *V. brasiliensis* pero que sean fértiles pasen a ser denominados *V. quadrangularis* Vell., admitiendo como válida esta especie. El nombre *V. brasiliensis* quedaría restringido a los ejemplares estériles por hibridez. El análisis de numerosos ejemplares de herbario permitió observar que en los mismos no se hallaron flores aberrantes o ausencia de clusas. Por lo tanto las sugerencias de Michael no se consideran.

El análisis del material tipo de *Verbena paucifolia* Turcz. no permite diferenciarlo de *V. litoralis* var. *bravibracteata*, por lo cual se considera un sinónimo de la misma.

Verbena boliviana Briq. se basa en un ejemplar del Herb. G: Bolivia, *Mandon 524* (Moldenke, 1942a: 428); debido a que carece de diagnosis constituye un nombre inválido que no debe validarse. El ejemplar se corresponde con *V. litoralis* var. *brevibracteata*.

Tipificaciones y nomenclatura. En 1818 Kunth funda *Verbena litoralis*; en 1825 Vellozo funda *V. brasiliensis*. Briquet (1904c: 292) combina *V. brasiliensis* como variedad de *V. litoralis*: *V. litoralis* var. *brasiliensis* (Vell.) Briq., y coloca *V. litoralis* como sinónimo de la misma; por lo tanto esta variedad no está válidamente publicada (Art. 26.2, Greuter et al., 2000) ya que contiene al tipo de *V. litoralis*. Munir (2002) validó este nombre (*V. litoralis* var. *brasiliensis* (Vell.) Munir) y presentó un correcto criterio para su separación de la variedad tipo, basándose en la inserción de las hojas al tallo y en caracteres florales y de las florescencias. Sin embargo, al existir la variedad *brevibracteata* de Kuntze (1894) ésta tiene prioridad (Art. 11, Greuter et al., 2000) por lo cual la variedad validada por Munir en 2002 es considerada sinónimo de la variedad *brevibracteata*.

Existe controversia en torno a la fecha de colección del tipo de *Verbena hanseni* Greene. J. Solomon (comun. pers.) de MO, opina que posiblemente la fecha 1898 publicada en el protólogo en Pittonia sería un error. El material en NDG tiene fecha anterior (1896) que la fecha publicada en Pittonia (1898) por lo cual se puede decir que es material original. Barbara J. Hellenthal (comun. pers.) de NDG señala que la letra en el ejemplar *V. hanseni*, NDG 043279, corresponde a E. L. Greene, "...la forma en que está escrito 'Greene' es especialmente típica de cómo el autor escribe su nombre...". Munir (2002) señala que el holotipo está en K, lo cual es posible ya que Greene visitó Kew y otros herbarios europeos en la década de 1890. Sin embargo, la Recomendación 9.4 A (Greuter et al., 2000) sugiere que el ejemplar de NDG se debería tomar como holotipo, por lo cual el ejemplar de K sería un isotipo.

Material adicional examinado. ARGENTINA. **Buenos Aires:** Pdo. Azul, *Ruiz Huidobro 1473* (SI); Pdo. San Isidro, Martínez, *Troncoso 19545* (SI); Pdo. Villarino, Ea. El Tigre, *Jørgensen 508* (BAB). **Chaco:** Dpto. Independencia, Avaí-Tavaí, *Pedersen 9867* (CTES, SI); Dpto. Mayor L. Fontana, Tacuruzal, de Villa Angela a Tostado, *Schulz 13745* (CTES). **Córdoba:** Dpto. Calamuchita, río Santa Rosa, *Krapovickas & Cristóbal 14692* (CTES); Dpto. Pumilla, Los Gigantes, *Sayago 989* (SI). **Corrientes:** Dpto. Bella Vista, Ao. Toropí, *Schinini 5361* (CTES, SI). **Entre Rios:** Dpto. Esquina, islas frente a Esquina, *Krapovickas et al. 26853* (CTES, SI). **Jujuy:** Dpto. Dr. M. Belgrano, camino de Reyes a lagunas de Yala, Abra Salvear, *Cabrera et al. 34553* (SI). **La Pampa:** Dpto. Rancul, entre Ing. Foster y La Maruja, *Troiani & Steibel 3919* (SI). **La Rioja:** Dpto. Chamical, sa. de los Llanos, *Biurrum & Pagliari 6408* (CTES). **Mendoza:** Dpto. Capital, co. de la Gloria, *Covas 568* (SI). **Neuquén:** Dpto. Minas, Anda Collo, *Rossow 2543* (BAB). **Río Negro:** Dpto. Avellaneda, Isla Choele-Choel, *Correa et al. 9709* (BAB, SI). **Salta:** Dpto. Candelaria, Agua Caliente, *Venturi 3702* (BAB, SI). **San Juan:** Dpto. Zonda, Zonda, *Múlgura & Deginani 104* (SI). **San Luis:** Dpto. Gral. Pedernera, co. de Las Cuevas, *Pastore 2034* (SI). **Santa Fe:** Dpto. La Capital, Sta. Fe, Col. Macías, 14 Mar. 1945, *Rotours s.n.* (BAB 66590). **Santiago del Estero:** Dpto. Robles, Lazaro, *Maldonado 453* (BA, SI). **Tucumán:** Dpto. Tafí, Los Chamicos, *Venturi 2767* (SI). BOLIVIA. **Cochabamba:** Prov. Chapara, Incachaca, *Steinbach 9681* (BA). BRASIL. **Santa Catarina:** Florianópolis, Ribeirão, *Klein & Bresolin 8410* (SI). CHILE. **Region Metropolitana:** Santiago, *Hicken 111* (SI). **II Región:** Antofagasta casa 1021 Chuquicamata, *Martin 176* (SI). **V Región:** Valparaíso, Coucón, *Boelcke 3921* (SI). **VII Región:** Linares, termas de Castillo, *Ricardi 2826* (SI). **VIII Region:** Prov. Bío Bío, Arauco, *Pedersen 14105* (CTES, SI). **X Región:** Valdivia, *Buchtien* Nro. Gay 22 (SI). PARAGUAY. **Alto Paraguay:** Chaco, Puerto Casado, *Rojas 2519* (SI). **Central:** San Lorenzo, *Basualdo 141* (FCQ). **Ñeembucú:** s. loc., 2 Mayo 1949, *Ramirez 349* (SI). **San Pedro:** Colonia Nueva Germania, *T. Rojas 1877* (SI), Loma Hoby, *Woolston 174* (SI). PERÚ. **Cuzco:** 3350 m s.m., *Herrera 17* (SI). ESTADOS UNIDOS DE AMÉRICA. **California:** Riverside Co., hills, *F. M. Reed 6029* (NY). **Massachusetts:** Wellesley College, 1 Oct. 1900, *s. coll. s.n.* (NY 14374). **South Carolina:** Jasper Co., *Mazzeo & Dudley*

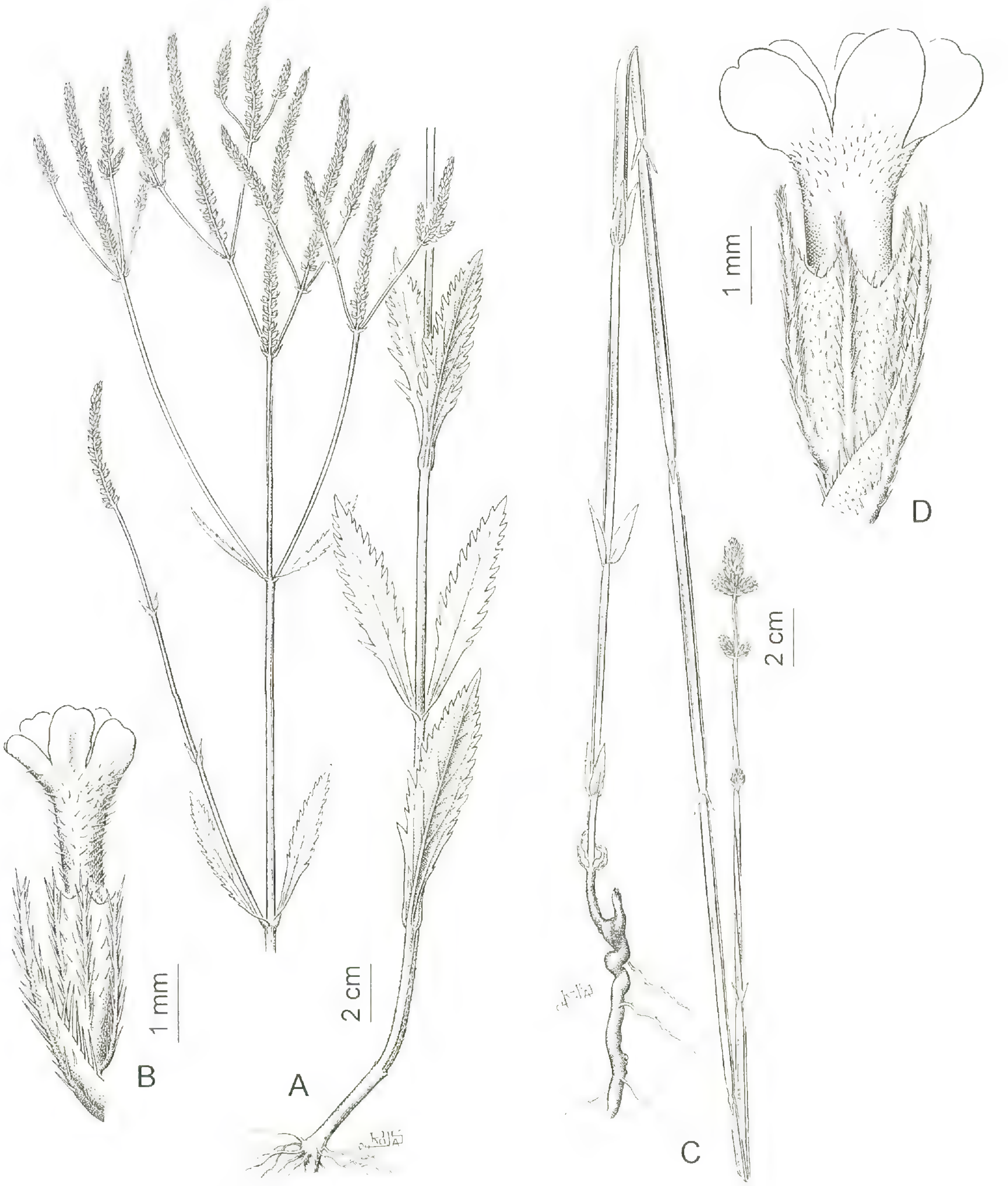


Figura 7. A, B. *Verbena litoralis* var. *brevibracteata*. —A. Aspecto general de la planta con inflorescencia. —B. Flor. C, D. *Verbena sagittalis*. —C. Aspecto general de la planta con inflorescencia. —D. Flor. A: de *Troncoso s.n.* (SI 19545); B: de *Rojas 1877* (SI); C: de *Múlgura 2252* (SI); D: de *Hagelund 10664* (SI).

1939 (BAB). **Texas:** Brazos Co., Wellborn, *Fryxell 1769* (CTES); Lamar Co., *Nee et al. 43959* (SI).

10. Verbena montevidensis Spreng., Syst. Veg. Ed. 16, 2: 747. 1825. TIPO: Uruguay. Montevideo: *Sellow* (holotipo, B†; isotipo, B foto F 17432!).

Verbena isabellei Briq., Annuaire Conserv. Jard. Bot. Genève 4: 234. 1900. Syn. nov. TIPO: Brasil. Rio Grande do Sul: *M. Isabelle 1835* (holotipo, G no visto; isotipos, G foto F 24690!, SI!).

Verbena minutiflora Briq. ex Moldenke, Phytologia 7: 84. 1959. Syn. nov. TIPO: Uruguay. Montevideo: *G. King 78* (holotipo, G no visto; isotipos, G foto F 24693!, SI!).

Verbena minutiflora var. *peruviana* Moldenke, Phytologia 50: 14. 1981. Syn. nov. TIPO: Perú. Cajamarca: Pampa de la Culebra, 1976, *A. J. Sagástegui Cabanillas S. & O. Dios C. 8385* (holotipo, TEX no

visto; isotipos, HUT no visto, MO no visto, TEX foto SI!, SI!).

Hierbas anuales o perennes, erectas, en ocasiones decumbentes, de 40–80 cm de altura, tallos subtetrágonos a cilíndricos en la base, lisos, glabros, gráciles hacia el ápice, ramificados. *Hojas* de lámina entera, de (1–)1.5–3(–7) × (0.3–)0.45–0.8(–2) cm, elípticas, anchas o angostas a casi lineares, reduciéndose en tamaño hacia el ápice de la planta, ápice agudo a subobtuso y base sésil cuneado-aguda, con los márgenes lisos o paucidentados hacia la mitad distal de la lámina, membranácea, no escariosa, subglabra o esparcidamente escabrosa sobre las poco evidentes venas del envés. *Florescencias* terminales, breves y delgadas, de 0.4–1(–2) × 0.45 cm en antesis, nunca mayores a 6 cm en fructificación, hipotagma presente, pedúnculos delgados, agrupadas en paracladios, los laterales superando la florescencia principal en fructificación; sinflorescencia bracteosa de más de un orden de ramificación; bráctea floral ovada, breve de (0.08–)0.15–0.18 (–0.27) cm, márgenes pilosos, siempre menor en longitud que el cáliz y adpresa al mismo. *Flores* sésiles poco evidentes, corola color lila o blanco; cáliz de (0.13–)0.2–0.24(–0.27) cm, con 5 dientes triangulares breves de ápice agudo, pubérulo apenas tomentoso; corola infundibuliforme, tubo corolino apenas exerto del cáliz, de (0.2–)0.3(–0.47) cm, lóbulos poco desarrollados; estambres inclusos en la mitad apical del tubo corolino. Clusas de 1.5 mm, dorso reticulado en la mitad superior, el resto estriado, cara comisural verrucosa. Anatomía caulinar tipo B.

Número cromosómico. $2n = 21$, $x = 7$, $3x = 21$ (Schnack & Covas, 1947).

Iconografía. Troncoso, 1979: 240, fig. 109; Lombardo, 1983: 54, lam. 20(2).

Distribución y ecología. Noreste de Argentina en la región mesopotámica y Buenos Aires; Paraguay, Uruguay y sur de Brasil hasta el estado de Paraná. Es una especie muy abundante, principalmente en pasturas de las provincias de Buenos Aires y Entre Ríos; el ganado se alimenta de las partes blandas como ser los ápices vegetativos y hojas jóvenes, razón por la cual es común encontrar ejemplares casi áfilos.

Observaciones. Verbena montevidensis es una especie muy variable en cuanto a la morfología de las hojas. Existen ejemplares con hojas anchamente elípticas de márgenes dentados (ej. *Krapovickas 20283*, SI!) hasta ejemplares con hojas angostamente elíptico-lineares de márgenes lisos (ej. *Pedersen 3831*, SI!; *Schinini et al. 7281*, SI!). *Verbena montevidensis* se asemeja a *V. litoralis* por la morfología de la inflorescencia y las hojas, por lo cual en muchas ocasiones han sido confundidos (Troncoso & Burkart, 1946), principalmente debido a que ambos taxones son muy variables en cuanto a la morfología foliar y al grado de ramificación de las sinflorescencias. *Verbena litoralis* se distingue por ser plantas de mayor altura, hasta de 2.5 m, tallos con pubescencia ligeramente escabrosa, florescencias más elongadas hasta de 18 cm y brácteas florales más largas, ca. 0.25 cm. Cuando las plantas de *V. montevidensis* están comidas por el ganado se pueden llegar a confundir con *V. ephedroides* ya que esta última presenta un aspecto vegetativo muy similar siendo más áfila (véase comentarios bajo *V. ephedroides*).

Moldenke (1959) al describir *Verbena minutiflora* menciona su afinidad con *V. montevidensis*, sin señalar las diferencias entre ambos taxones; solamente refiere como característico de *V. minutiflora* hojas lineales y florescencias breves. Se han observado ejemplares que tienen florescencias breves como *V. minutiflora* y hojas semejantes a *V. montevidensis* (ej. *Galli 141*, SI!), otros con hojas lineares y florescencias como *V. montevidensis* (ej. *Hagelund 6525*, SI! y *Reitz & Klein 12551*, SI!). Consecuentemente, los caracteres anteriormente mencionados no permiten la separación de ambos taxones.

Moldenke (1981: 14) distingue *Verbena minutiflora* var. *peruviana* de la variedad tipo por su menor altura y hábito decumbente, y por la pubescencia de las florescencias, siendo ambos caracteres muy variables en este taxón por lo cual no tienen relevancia taxonómica.

La posición taxonómica de *Verbena isabelli* Briq. ha sido controversial. Así, Moldenke (1942a: 432) la consideró sinónimo de *V. minutiflora*, y en un trabajo posterior (Moldenke, 1964b: 159) la consideró sinónimo de *V. montevidensis*. Munir (2002) la incluye bajo la sinonimia de *V. litoralis* var. *brasiliensis*, pero las hojas del tipo de *V. isabelli* son de márgenes enteros, muy similares a *V. montevidensis*, y las flores son pequeñas para ser *V. litoralis*. El análisis del material tipo y de la descripción original de *V. isabelli* permitió determinar que no existen diferencias morfológicas con *V. montevidensis*, razón por la cual se incluye a la variedad bajo la sinonimia de este último taxón.

Material adicional examinado. ARGENTINA. **Buenos Aires:** Pdo. Tigre, Isla M. García, ca. arenal, *Hurrel et al. 2116* (SI); Pdo. Lobos, Lobos, *Burkart 27701* (SI). **Córdoba:** Dpto. Unión, Bell Ville, la Rinconada, *Ferrucci 186* (CTES). **Corrientes:** Dpto. Capital, Perichón, *Krapovickas et al. 26513* (CTES, SI); Dpto. Mercedes, Mercedes, *Schinini et al. 7281* (CTES), Ea. Itá Caabó, *Pedersen 3831* (SI); Dpto. San Martín, Pellegrini, arrocera Drews, *Krapovickas 20283* (SI). **Entre Ríos:** Dpto. Concordia, Colonia Yeruá, *Galli 141* (SI); Dpto. Villaguay, Jubileo, *Pedersen 6309* (CTES, SI). **Misiones:** Dpto. Caingúas, predio UNLP Valle del A° Cuña

Pirú, *Biganzoli et al. 298* (LP, SI); Dpto. Iguazú, ruta 12, *Krapovickas & Cristóbal 15613* (CTES, LP, SI). **San Luis:** Dpto. Belgrano, Los Araditos, *D. Lee-Anderson 3108* (CORD). **Santa Fe:** Dpto. San Martín, 5 km N de San Jorge, *M. Astegiano 8* (CORD). BRASIL. **Paraná:** Serra Negra, Guaraqueçaba, *Hatschbach 24883* (SI). **Rio Grande do Sul:** Santo Angelo, *Hagelund 6525* (SI); Pque. Sta. Rita, Farroupilha, *Bueno 1109* (CTES). **Santa Catarina:** Serril, Capoeira, *Reitz & Klein 12551* (SI); Fraiburgo, gruta, *Krapovickas & Cristóbal 43960* (CTES). PARAGUAY. **Boquerón:** Picada Tte. Ochoa, *Basualdo 843* (FCQ). **Caaguazú:** ruta 2 km 98, *Zardini & Aguayo 10547* (FCQ, MO, SI). **Caazapá:** Tavai, *Degen 1729* (FCQ). **Central:** Carapeguá, *Degen 1436* (FCQ). **Cordillera:** Piraretá, E, *Lurvey 253* (PY). **Guairá:** Tororo, *Degen 1070* (FCQ). **Itapúa:** Pirapo, 18 Dic. 1985, *Yosisaki s.n.* (FCQ). **Misiones:** San Ignacio, *Basualdo 720* (FCQ). **Paraguarí:** estero de Ypoá, *Zardini & Velásquez 25220* (AS, MO, SI). URUGUAY. **Canelones:** Sta. Lucía, *Felippone 6935* (SI). **Cerro Largo:** Río Negro, Ea. Palleros, *Gallinal et al. 1611* (SI). **Florida:** río Yi y Ao. Mansavillagra, *Rosengurtt B730* (SI). **Montevideo:** Miguelete, *Herter 269* (SI). **Paysandú:** camino Sacra, *Felippone 3155* (SI); Montevideo, *Rimbeth 20* (SI). **Salto:** Termas de Acapey, *Pedersen 16189* (CTES).

11. Verbena ovata Cham., Linnaea 7: 263. 1832. TIPO: Brasil, *F. Sellow* (lectotipo, designado por O'Leary (2006: 497), K no visto; isotipos, BR foto SI!, K foto SI!, SI!). Figura 8.

Verbena bonariensis L. f. *robustior* Chodat, Bull. Herb. Boissier, sér. 2(2): 817. 1902. Syn. nov. TIPO: [Paraguay.] "In palude pr. Igatimi", Sep. 1898 1899, *Hassler 4695* (lectotipo, aquí designado, G no visto; isotipo, G foto SI!).

Hierbas perennes, hasta de 1.6 m de altura, ramas y tallos erectos, no muy ramificados, hojosos en la base con entrenudos breves; subáfilos hacia el ápice con entrenudos distanciados; pubescencia de pelos patentes poco densos. *Hojas* de lámina entera, de 5–6(–9) × 3–4.5(–5.5) cm, generalmente ovada, ápice obtuso a agudo, sésiles de base subauriculada, los márgenes dentado-serrados a lo largo de toda la lámina a excepción de la parte basal, dientes irregulares acuminados, textura coriácea-escariosa, con prominente venación en envés, ásperas al tacto con abundantes pelos escabrosos; las hojas apicales de la planta inconspicuas, escamosas pero similares a las basales en cuanto a textura y venación. *Florescencias* terminales, cilíndricas, densas, breves y anchas de 1–2 × 0.8–1 cm en antesis, alargándose en fructificación, la principal sésil, las laterales brevemente pedunculadas, nunca superándola, agrupadas en paracladios multímeros en torno a la florescencia principal dando el aspecto de inflorescencia corimbosa compacta; sinflorescencia bracteosa, mayor a un orden de ramificación; brácteas florales largas, de 0.5–0.7 cm, elíptico-angostas de ápice agudo, siempre sobrepasando al cáliz en longitud y sobresaliendo de la florescencia, pubescencia espaciadamente adpresa, los márgenes pilosos. *Flores* sésiles, corola color lila, violácea, a veces azul, purpúrea; cáliz de 0.32–0.34 (–0.45) cm, con 5 dientes triangulares de ápice agudo, escasamente adpreso pubescentes; corola infundibuliforme, tubo corolino largo de 0.8–1(–1.2) cm, pubescencia vilosa externa, estambres superiores con el conectivo apiculado. Clusas de 1.8–2 mm, dorso reticulado en el tercio superior, el resto estriado, cara comisural verrucosa. Anatomía caulinar tipo B.

Número cromosómico. $2n = 72$ (Noack, 1937 considera que es $10x + 2$).

Distribución y ecología. Noreste de Argentina en Misiones y Corrientes, este de Paraguay y sur de Brasil. Habita en campos pantanosos o bajos.

Observaciones. *Verbena ovata* es fácilmente reconocible por sus florescencias densas, subglabras, con pelos adpresos, nunca con pelos glandulares y las brácteas florales largas, elíptico-angostas de ápice agudo, sobresaliendo de las florescencias. El análisis del material de herbario permitió determinar que existen ejemplares con hojas elíptico-lineares en vez de ovadas (ej. *Hagelund 10167*, SI!; *Sehnem 5598*, SI!), pero el resto de los caracteres tanto vegetativos como florales se corresponden con este taxón, por lo cual la denominación "*ovata*" no refleja una característica del taxón. *Verbena ovata* es morfológicamente afín a *V. bonariensis*, compartiéndo el tipo de inflorescencia, densa, de aspecto corimboso (véanse diferencias entre estas dos especies bajo observaciones de *V. bonariensis*).

Verbena bonariensis f. *robustior* Chodat se basa en tres colecciones de *Hassler* (sintipos): *3324*, *3852* y *4695*. Los tres ejemplares citados se corresponden actualmente con tres taxones diferentes: el ejemplar *Hassler 3852* se corresponde con *V. litoralis* var. *brevibracteata* (Kuntze) N. O'Leary, el ejemplar *Hassler 3324* se corresponde con *V. intercedens* Briquet, y el ejemplar *Hassler 4695* se designa lectotipo de la forma *robustior* Chodat. Este último ejemplar se corresponde con el protólogo y con el ejemplar tipo de *V. ovata* Cham. por lo cual se trata la forma *robustior* como sinónimo de *V. ovata*.

Material adicional examinado. ARGENTINA. **Corrientes:** Dpto. Sto. Tomé, Sto. Tomé, ca. Col. Garabí, *Krapovickas et al. 17069* (BAB, CTES, LP); Ea. Garrucho, *Guaglianone et al. 232* (SI). **Misiones:** Dpto. Candelaria, Sta. Ana, *Rodriguez 596* (BA, BAF, SI); Dpto. Apóstoles, Pindapoy, *Bridarolli 3024* (LP); Co. Sta. Ana, *Niederlein 541* (SI). BRASIL. **Paraná:** Rio Chopim, Mun. Palmas, *Hatschbach 28218* (SI). **Rio Grande do Sul:** Osorio, Lagoa das Malvas, *Sehnem 5598* (SI); Cruz Alta, *Hagelund 10168* (CTES); Cruz Alta, *Hagelund 10167* (SI). **Santa Catarina:** Tupitinga, *Reitz & Klein 14409* (SI). PARAGUAY. **Alto Paraná:** 1909/10, *Fiebrig 5645* (SI). **Amambay:** ca. Cap. Bado, *Schinini et al. 35453* (CTES). **Caazapá:** San Juan

Nepomuceno, *Rojas 5788* (SI); Tavai, *Degen 1304* (FCQ). **Itapúa:** Yacyretá, *Zardini & Gamarra 59262* (AS, MO, SI). **Paraguarí:** Cord. Ibytymí, *Rojas 6128* (SI). **San Pedro:** 29 Nov. 1957, *Woolston 916* (SI).

12. Verbena rigida Spreng., Syst. Veg. Ed. 16, 4(2): 230. 1827. *Verbena bonariensis* var. *rigida* (Spreng.) Kuntze, Revis. Gen. Pl. 3(2): 255. 1894. TIPO: Brasil. Rio Grande do Sul: s. loc., *F. Sellow 428* (lectotipo, designado por Munir (2002: 39), VT no visto; isotipos, P no visto, SI!).

Verbena venosa Gillies & Hook. ex Hook., Bot. Misc. 1: 167. 1829. *Verbena bonariensis* f. *venosa* (Gillies & Hook. ex Hook.) Voss, Vilm. Blumengärtn. ed. 3(1): 826. 1894. *Verbena bonariensis* var. *venosa* (Gilles & Hook. ex Hook.) Chodat, Bull. Herb. Boissier, sér. 2(2): 817. 1902. TIPO: [Argentina.] Buenos Aires: *J. Gillies s.n.* (holotipo, K no visto; isotipo, K foto SI!, SI!).

Verbena scaberrima Cham., Linnaea 7: 267. 1832. TIPO: Brasil, *F. Sellow* (neotipo, designado por Munir (2002: 39), P 00289952 no visto; isotipos, K no visto, NY foto SI!, P foto SI!, SI!).

Verbena scabra Marnock, Floric. Mag. & Misc. Gard. 5: 87. pl. 54, fig. 3. 1840, nom. illeg., non *Verbena scabra* Vahl, Eclog. Amer. 2: 2. 1798. TIPO: lámina de Floric. Mag. & Misc. Gard. 5: 87, pl. 54, fig. 3. 1840 (lectotipo, designado por O'Leary (2006: 498)).

Verbena doniana Steud., Nomencl. Bot. Ed. 2(2): 750. 1841. Nombre reemplazado: *Verbena rugosa* D. Don, in Sweet Brit. Fl. Gard. 7(2,4): pl. 318. 1836, nom. illeg., non *Verbena rugosa* Mulh. ex Willd., Enum. Hort. Berol. 633. 1809. TIPO: lámina de Brit. Fl. Gard. 7(2,4): pl. 318. 1836 (lectotipo, designado por O'Leary (2006: 497)).

Verbena venosa var. *reineckii* Briq., Annuaire Conserv. Jard. Bot. Genève 3: 164. 1899. *Verbena rigida* var. *reineckii* (Briq.) Moldenke, Phytologia 2: 151. 1946. Syn. nov. TIPO: Brasil. Rio Grande do Sul: Belem Velho, 31 Oct. 1897, *E. M. Reineck & Czermak 55* (holotipo, G no visto).

Verbena rigida f. *obovata* Hayek, Bot. Jahrb. Syst. 42: 162. 1908. *Verbena rigida* var. *obovata* (Hayek) Moldenke, Revista Sudamer. Bot. 5: 2. 1937. Syn. nov. TIPO: Paraguay. "In viciniis Caagauzú", 1905, *E. Hassler 8911* (holotipo, G no visto; isotipos, G foto SI!, NY foto SI!, SI!, W foto SI!).

Verbena rigida var. *glandulifera* (sphalm. *glandulosa*) Moldenke, Phytologia 6: 331. 1958. Syn. nov. TIPO: Brasil. Paraná: Laranjeiras do Sul, 15 Nov. 1957, *G. Hatschbach 4212* (holotipo, TEX no visto; isotipos, SI!, TEX foto SI!).

Verbena rigida f. *paraguayensis* Moldenke, Phytologia 34: 20. 1976. Syn. nov. TIPO: Paraguay. Carapegná, Feb. 1919, *T. Rojas 3407* (holotipo, M no visto; isotipos, M foto SI!, SI!, TEX foto SI!).

Hierbas o sufrútices, rizomatosas o estoloníferas, hasta de 60 cm de altura, tallos decumbentes en la base, simples o ramificados, híspido-escabrosos con algunos pelos glandulares. *Hojas* de lámina entera, de (3–)7.5–10(–12) × 1–2.5(–3.5) cm, oblongo-elípticas u obovadas, ápice agudo o subobtuso, sésiles, de base subauriculada, los márgenes marcada y uniformemente inciso-serrados, dientes agudos de senos cóncavos, generalmente coriáceas algo escariosas, rígidas, muy ásperas al tacto con pelos escabrosos, con venas principales impresas prominentemente en el envés, venación secundaria poco notoria. *Florescencias* terminales, densas y congestas cuando jóvenes, luego cilíndricas y elongándose a la madurez, de 2–5(–10) cm, reunidas en paracladios trímeros laxos, la principal sésil y las laterales brevemente pedunculadas superando a la principal, sinflorescencias bracteosas generalmente de más de un orden de ramificación; pubescencia escabrosa y densamente glandular principalmente en los pedúnculos, brácteas florales ovado-angostas de 0.45–0.6 cm, mayores que el cáliz, de ápice acuminado y márgenes ciliados, rígidas. *Flores* sésiles, corola color lila, rosa, violácea, purpúrea o blanca; cáliz de 0.3–0.35 cm con 5 dientes triangulares de ápice agudo, con pubescencia hirsuto-glandular, en ocasiones de coloración rojiza, glanduloso-pubescentes; corola hipocraterimorfa, tubo corolino delgado y largo de (0.5–)0.65–0.9(–1.1) cm, sobresaliendo de la florescencia, muy vistoso, exteriormente piloso, limbo corolino desarrollado, ca. 0.7 cm diámetro, algunos lóbulos ligeramente bífidos; estambres insertos en la mitad inferior del tubo corolino, muchas veces con el conectivo apiculado. Clusas de 1.5–2 mm, dorso reticulado en el tercio superior, el resto estriado, cara comisural papilosa. Anatomía caulinar tipo A.

Iconografía. Schnack y Covas, 1946: 282, fig. 1c (clusa); Troncoso, 1979: 235, fig. 106; Everett, 1982: 3482 (foto); Botta y Cabrera, 1993: 96, fig. 43; Munir, 2002: 43, fig. 2; Méndez Santos, 2003: 10, fig. 1.

Distribución y ecología. Nativa de Sudamérica, crece en Uruguay, sur de Brasil, este de Paraguay, sur de Bolivia y norte y noreste de Argentina, alcanzando su límite sur de distribución en el norte de la provincia de Buenos Aires. Está naturalizada en el resto de América (Estados Unidos de América, Centroamérica), en parte de Europa, Sudáfrica y este de Asia. Munir (2002) la cita para el sudeste de Australia, como introducida desde Sudamérica. Crece en tierra negra fértil, en pasturas arenosas, en orilla de monte pedregoso, en campos con rocas aflorantes, en bañados, en campo sobrepastoreado, quemado y erosionado, al costado de ruta o en la selva marginal sobre arroyos; solitaria o formando grandes matas.

Observaciones. Verbena rigida es muy fácilmente diferenciable por sus hojas con los márgenes

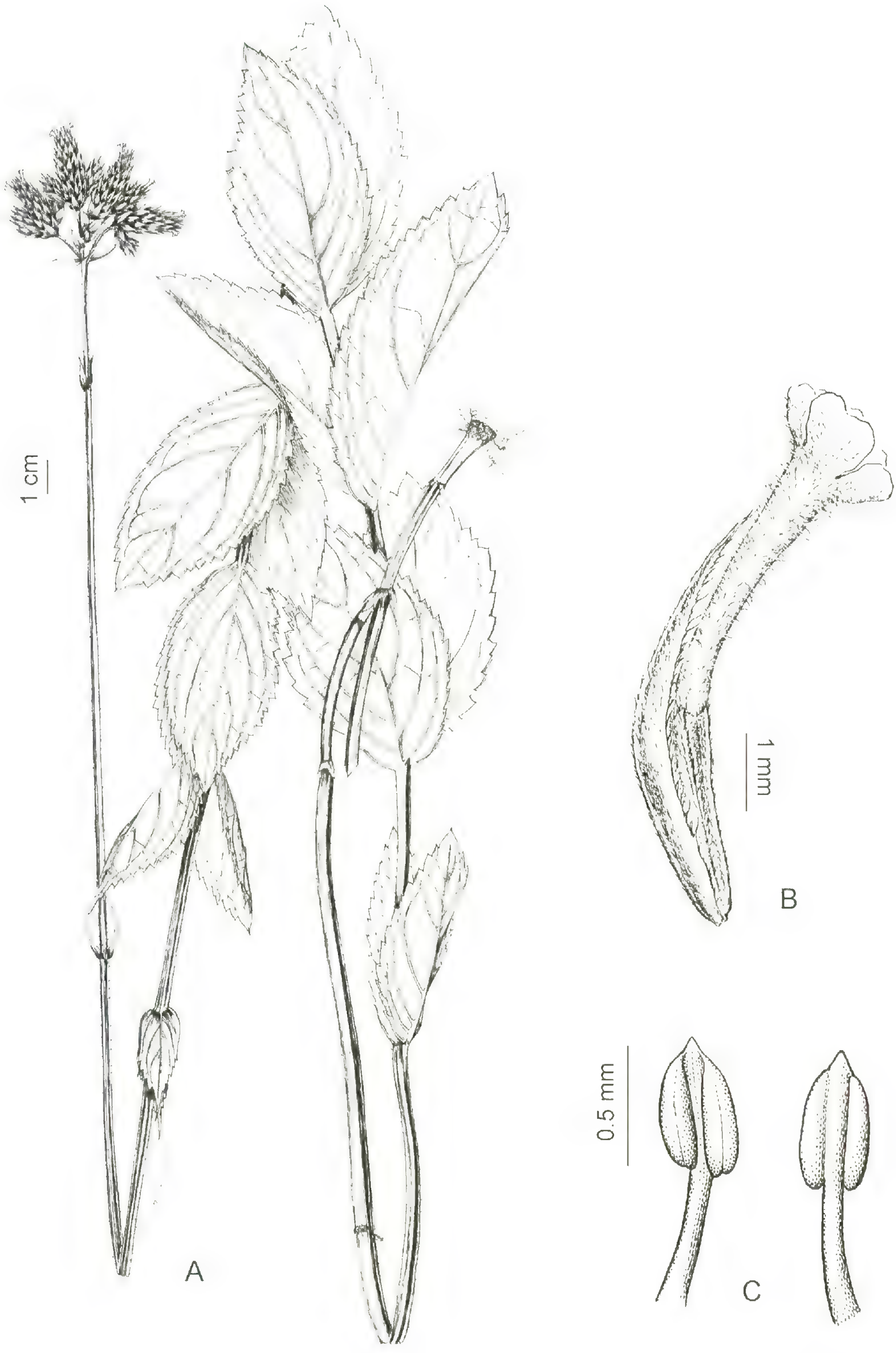


Figura 8. *Verbena ovata.* —A. Aspecto general de la planta con inflorescencia. —B. Flor. —C. Estambres superiores vista dorsal y ventral. A, C: de *Guaglianone 232* (SI); B: de *Rodriguez 596* (SI).

marcadamente inciso-serrados, con dientes agudos de senos cóncavos. Sin embargo puede llegar a confundirse con *V. intermedia* o *V. intercedens* (véanse comentarios bajo estas dos últimas especies). Este taxón presenta caracteres frecuentes en las especies del género *Glandularia* (Botta, 1989) como ser la corola hipocraterimorfa y el largo del tubo corolino, por lo cual muchas veces ha sido determinada como *G. phlogiflora* (Cham.) Schnack & Covas, *G. canadensis* (L.) Nutt. o simplemente *Glandularia* indet. Sin embargo el largo del estilo y la morfología de las clusas la acercan a *Verbena*. El número cromosómico sería un buen carácter para resolver a que género corresponde, siendo un número básico $x = 5$ característico de *Glandularia* y un número básico $x = 7$ de *Verbena* (Lewis & Oliver, 1961). Hasta el momento se cuenta con el dato del número cromosómico diploide $2n = 42$ (Junell, 1934; Dermen, 1936), que representaría un número básico $x = 7$. Por otro lado, este taxón presenta anatomía caulinar tipo A, lo cual es frecuente en *Glandularia* (Botta, 1989).

Hayek (1908: 162) distingue a *Verbena rigida* f. *obovata* por tener las hojas basales ligeramente más anchas y obovadas que la forma tipo. A partir del análisis de numeroso material de herbario se encontraron ejemplares con ambos tipos de hojas en la misma planta (ej. *Ahumada & Eskucke 3218*, SI!; *Burkart 1430*, SI!), consecuentemente se considera la forma *obovata* bajo la sinonimia de *V. rigida*.

Moldenke (1976b: 20) señala que la forma *paraguayensis* difiere de la variedad tipo por la pubescencia hirsuta. Por otro lado, la variedad *glandulifera* de Moldenke (1958: 331) difiere de la variedad tipo por la mayor densidad de pubescencia glandular. La pubescencia es un carácter muy variable dentro de *V. rigida*, hallándose ejemplares con abundante pubescencia (ej. *Zardini 8706*, SI!; *Cabrera 28475* SI!) y otros escasamente pubescentes (ej. *Burkart 1430*, SI!), por lo que este carácter no permite diferenciar taxones infraespecíficos.

Verbena rigida var. *lilacina* (Benary & Bodger ex G. Harrow) Moldenke y variedad *alba* (Benary & Bodger ex G. Harrow) Moldenke se diferenciarían de la variedad tipo en el color de las corolas, azul-lavanda casi celeste y blanca, respectivamente. Moldenke (1964d: 96) menciona que el color de las flores de *V. rigida* puede presentar una amplia gradación de tonos dentro de la gama de los violetas, lilas, azules y celestes. El estudio de abundante material de herbario pudo demostrar que el color de las flores es un carácter variable que carece de valor taxonómico.

Briquet (1899: 164) distingue a *Verbena venosa* var. *reineckii* por la textura membranácea, no escabrosa de las hojas. Esta característica se observó en muchos de los ejemplares que crecen en la sombra (ej. *Cabrera & Troncoso 29279*, SI!; *Schinini 3455*, SI!; *Hagelund 132-40*, SI!); motivo por el cual no se sustenta la variedad dado que se trataría de una morfología ambiental umbrófila.

Verbena rigida es una planta muy vistosa usada como ornamental.

Material adicional examinado. ARGENTINA. **Buenos Aires:** Dpto. Campana, Campana, *Nicora 1988* (SI). **Chaco:** Dpto. Bermejo, Campo Vélaz, *Schulz 17688* (CTES). **Córdoba:** Dpto. Punilla, Casas viejas, entre Olaen y Pampa de San Luis, *F. Kurtz 10457* (CORD); Dpto. Calamuchita, sa. Gde., falda E, *A. Hunziker 15934* (CORD). **Corrientes:** Dpto. Berón de Astrada, *Schinini 13995* (CTES, SI); Dpto. Ituzaingó, ruta 12, *Ahumada & Eskucke 3218* (SI); Dpto. Saladas, Ea. Tatacua, *Hauman 23540* (BA); Dpto. San Luis del Palmar, *Cristóbal et al. 1281* (CTES, SI). **Entre Ríos:** Dpto. Diamante, Crespo, *Burkart & Troncoso 28060* (SI). **Formosa:** Dpto. Laishi, reserva El Bagual, *Di Giacomo 448* (CTES). **Jujuy:** Dpto. Dr. M. Belgrano, s.d., *Lynch s.n.* (SI 3848). **Misiones:** Dpto. Apóstoles, Apóstoles, *Cabrera 28475* (SI), Apóstoles, *Cabrera & Troncoso 29279* (SI); Dpto. Candelaria, Loreto, Est. Agronomía, *Burkart 1430* (SI); Dpto. Lib. Gral. San Martín, Pto. Mineral, *J. E. Montes 802* (BAB, CTES); Dpto. San Pedro, Piray-Guazú, *Montes 4308* (LP); Dpto. Sta. Ana, s. loc., *Rodriguez 545* (BA, SI). **Santa Fe:** Dpto. Gral. Obligado, Chaco Santafesino, Mocoví, *Venturi 5* (BA, SI). **Tucumán:** Dpto. Burruyacú, Sa. de Muria, *Venturi 273* (SI). BOLIVIA. **Santa Cruz:** Co. Alta Mairana, *Steinbach 6052* (SI); Prov. Sta. Cruz, A Samaipata, El Fortín, *J. Hunziker & M. D. Hunziker 13223* (SI). BRASIL. **Paraná:** Curitiba, Vila Tingui, *Hatschbach 33830* (LP); Colombo, rio Palmital, *Hatschbach 32787* (LP); Guarapuava, *Reitz & Klein 17672* (SI). **Rio Grande do Sul:** Sta. Barbara do Sul, *Krapovickas & Cristóbal 33724* (CTES); Granja Sodal, *Hagelund 132-40 N°960* (SI). **Santa Catarina:** Bom Jardim da Serra, *Ahumada & Eskucke 2963* (SI); Florianópolis, *Klein & Bresolin 8437* (SI); Marombas, *Klein 3286* (SI). **São Paulo:** Alto Bananeiras, *Arbo et al. 7734* (CTES). PARAGUAY. **Alto Paraná:** 1909/10, *Fiebrig 6305* (SI). **Caaguazú:** Palomares, *Schinini & Caballero Marmori 30051* (CTES); entre Estigarribia y M. Frutos, *Morrone & Pensiero 302* (FCQ). **Caazapá:** Tavai, *Degen 815* (FCQ). **Cordillera:** Salto Piraretá, *Harley et al. 28030* (CTES, FCQ). **Guairá:** Villarica, *Jørgensen 3770* (LP, SI). **Misiones:** San Juan Bautista, *E. Lurvey 39* (PY). **Paraguarí:** Pque. Nac. Ibicuyí, *Zardini 8706* (PY; SI); Acahay, *Schinini 3455* (SI); Pque. Nac. Ibicuyí, *Zardini 8706* (PY, SI); Ibytimi, *Schinini 3453* (CTES, SI). URUGUAY. **Canelones:** Pedrera, *Rosengurtt B1920* (SI); La Estanzuela, *Burkart 15773* (SI). **Lavalleja:** Minas, *Osten 16049* (BA); ca. villa Serrana, *Solis Neffa et al. 288* (CTES). **Maldonado:** Piriápolis, *Tremoleras s.n.* (SI 3849). **San José:** Sa. Mahoma, *Krapovickas & Cristóbal 16245* (CTES). **Tacuarembó:** Pozo Hondo, *Cabrera & Zuloaga 32353* (SI).

JAMAICA: Rd. to Salt Hill, *Harris 11969* (SI).

AUSTRALIA: Gloucester, the big rock, *Coveny 16706* (CTES).

13. Verbena sagittalis Cham., Linnaea 7: 259. 1832. TIPO: Brasil. Rio Grande do Sul: 1833, *C. Gaudichaud 521* (neotipo, designado por O'Leary (2006: 497), P 00289956 no visto; isotipo, P foto SI!). Figura 7C, D.

Verbena lindmanii Briq., Ark. Bot. 2(10): 12, tab. 3A. 1904. Syn. nov. TIPO: Brasil. Rio Grande do Sul: Cachoeira, 1893, *C. A. M. Lindman A1203* (holotipo, S no visto; isotipos, NY foto SI!, S foto SI!, SI!).

Sufrútices de 0.45–1 m de altura, raiz leñosa engrosada y vertical de la cual surgen raices laterales delgadas, ramas tetrágonas longitudinalmente estriadas, glabras, en ocasiones levemente aladas con los nudos algo angostados, muy distanciados hacia el ápice (entre 4–8 cm), de aspecto áfilo. *Hojas* de lámina entera, pequeñas de 1–2.5 × 0.5 cm, elíptico-ovadas, ápice obtuso, subobtuso o agudo, sésiles de base redondeada, los márgenes lisos a subcrenado-dentados, subglabras a esparcidamente estrigosas en ambas caras. *Florescencias* terminales, cilíndricas, breves, de 2.5(–4) × 0.8 cm, hipotagma ausente, florescencias sésiles, agrupadas de a tres, en la axila de hojas caducas escamosas, sinflorescencia con ramificaciones de primer orden; brácteas florales de (0.25–)0.3–0.35(–0.4) cm, ovadas, ápice agudo, marginalmente ciliadas, generalmente menores en longitud que el cáliz, finamente estrigoso pubescentes. *Flores* sésiles de corola color azul, celeste o lila; cáliz de (0.35–)0.4–0.5 cm, con 5 dientes largos subulados acuminados, subglabro entre costillas, esparcidamente estrigoso sobre las mismas; corola infundibuliforme, tubo corolino ca. 0.55 cm, pubescente en la garganta, pelos moniliformes hacia la fauce, limbo poco desarrollado; estambres insertos en la mitad del tubo corolino. Clusas de 2–2.5 mm, dorso reticulado en el tercio superior, el resto estriado, cara comisural muricada. Anatomía caulinar tipo B.

Iconografía. Briquet, 1904b: 2(10): 12, tab. 3A (sub nom. *Verbena lindmanii* Briquet).

Distribución y ecología. Argentina: Misiones, Brasil: Paraná, Santa Catarina y Rio Grande do Sul. Habita en campos secos a orillas de montes, es escasa.

Observaciones. Verbena sagittalis es fácilmente reconocible por la ausencia completa de desarrollo del hipotagma de la inflorescencia, siendo las florescencias sésiles surgiendo todas de un mismo punto del pedúnculo de la sinflorescencia.

Tipificaciones y nomenclatura. La descripción de *Verbena lindmanii* en el protólogo (Briquet, 1904b: 12) coincide con lo que Chamisso (1832: 259) denominó *V. sagittalis* alrededor de 70 años antes. Briquet define su especie como un sufrútice de ramas glabras, entrenudos largos, hojas pequeñas subescamosas hacia el ápice y florescencias congestas; todas características que también distinguen a *V. sagittalis*. Además, la ilustración de *V. lindmanii* de Briquet (1904b: tab. 3) refleja la similitud con *V. sagittalis*. Briquet (1904b: 12) señala que *V. lindmanii* sería afín a *V. sagittalis* compartiendo con ésta el tipo de inflorescencia y de flores; distingue a *V. lindmanii* porque los frutos se separan en 4 clusas solo bajo presión. El estudio de material permitió evidenciar que este carácter depende mucho del tiempo de maduración del fruto y por lo tanto no puede considerarse de valor taxonómico. Consecuentemente en el presente trabajo se incluye a *V. lindmanii* bajo la sinonimia de *V. sagittalis*.

Material adicional examinado. ARGENTINA. **Misiones:** Dpto. Candelaria, Sta. Ana, 27 Ene. 1907, *Spegazzini s.n.* (BAB 17626); Loreto, *Cabral et al. 323* (CTES); Yabebyry, *Montes 15076* (SI). BRASIL. **Paraná:** Vila Velha, *Krapovickas & Cristóbal 40873* (CTES); Pta. Grossa, Vila Velha, *Hatschbach 2896* (SI); Palmeira, *Hatschbach 20157* (SI). **Rio Grande do Sul:** Vacaría, *Pedersen s.n.* (CTES 354599); Morro Santana, Porto Alegre, *Soares 242* (CTES); Arroio dos Ratos, Faz. Faxinal, *Hagelund 14314* (CTES); Torres, *Hagelund 7659* (SI); Granja Faxinal, Arroio dos Ratos, *Hagelund 10664* (SI). **Santa Catarina:** Curralindo, Araranguá, *Reitz c874* (SI); Campos Novos, *Reitz 6422* (SI).

II. Verbena serie **Pachystachyae** subserie **Pseudoracemosae** N. O'Leary, subser. nov. TIPO: *Verbena filicaulis* Schauer.

Herba vel suffrutex, florescentia pauciflora. Transectio caulina cum parenchymate clorophylliano continuo.

La subserie *Pseudoracemosae* está conformada por 5 especies y 2 variedades; agrupa todos aquellos taxones con transcorte caulinar que evidencia disposición del parénquima clorofiliano de manera continua (Fig. 2C) y florescencias con flores no densamente imbricadas, espaciadas más de 2 mm. *Verbena caniuensis* Moldenke, posee flores pediceladas, lo cual semejaría un pseudoracimo, de ahí el nombre de la subserie.

Las especies de esta subserie poseen una distribución más restringida que las de la subserie *Pachystachyae*, encontrándose únicamente en el sur de Brasil en los estados de Rio Grande do Sul, Santa Catarina, Paraná hasta São Paulo y en el noreste de Argentina en la provincia de Misiones.

14. Verbena caniuensis Moldenke, Phytologia 4: 128. 1952. TIPO: Brasil. Paraná: Palmeira, Rio Caniú, 11 Nov. 1951, *G. Hatschbach 2560* (holotipo, NY no visto; isotipos, NY foto SI!, SI!). Figura 9A–C.

Hierba rastrera, procumbente, pequeña hasta de 25 cm de altura; tallos tetrágonos, entrenudos de 2.5–4 cm; ramas procumbentes con raíces adventicias en los nudos; pubescencia subglabra a ligeramente pilosa con pelos adpresos blanquecinos. *Hojas* de lámina entera, suborbicular, de 1–2 cm, ápice obtuso, base

redondeada, atenuada hacia el pecíolo, éste frágil y largo de 1–2 cm, los márgenes dentado-crenados, la cara abaxial marcadamente reticulado-nervosa glabra a excepción de las venas, cara adaxial con pelos adpresos breves espaciadamente dispuestos. *Florescencias* terminales, solitarias, laxas, de 7–13 cm con flores espaciadamente dispuestas, pedúnculo delgado de 4–4.5 cm; brácteas florales ovadas, breves de 0.15–0.3 cm, generalmente menores en longitud que el cáliz, subglabras, los márgenes poco pilosos. *Flores* de corola color lila, con breve pedicelo que se alarga hasta 1.5 mm después de la antesis; cáliz de 0.2–0.3 cm, con 5 dientes triangulares breves de ápice agudo, escasa pubescencia adpresa principalmente sobre las costillas; corola infundibuliforme, tubo corolino apenas exerto del cáliz, de 0.43 cm, fauce pilosa, estilo breve. Clusas de 1.8–2 mm dorso liso ligeramente estriado, cara comisural lisa. Anatomía caulinar tipo A.

Distribución y ecología. Endémica de Brasil del estado de Paraná. Se la ha encontrado en terreno húmedo.

Observaciones. Verbena caniuensis se distingue por su hábito rastrero con raíces adventicias surgiendo de cada nudo, sus hojas largamente pecioladas de lámina orbicular y por las florescencias solitarias con flores brevemente pediceladas. Este último carácter la aleja de *Verbena* s. str. y la acercaría a otros géneros como *Tamonea* Aubl., que posee flores brevemente pediceladas. La inflorescencia de *V. caniuensis* posee un patrón de ramificación particular donde de la axila de las dos hojas basales de la florescencia se desarrollan ramas laterales, con hojas, que también rematan en una florescencia, y así sucesivamente. Muchas veces una de esas yemas laterales no desarrolla y la planta crece solo por una de las ramas laterales, por lo cual las florescencias poseen un aspecto axilar. Las florescencias podrían ser monobotrios o pleiobotrios dependiendo de si la iniciación de las florescencias nuevas se considera proléptica o siléptica respectivamente (sensu Weberling, 1992: 254). Dado que en *Verbena* las florescencias siempre son pleiobotrios (Martínez et al., 1996), lo más probable sería que se tratase de un pleoibotrio frondoso, es decir, que la iniciación de las nuevas florescencias fuera siléptica.

Material adicional examinado. BRASIL. **Paraná:** Palmeira, Rio Caniú, *Hatschbach 20177* (CTES, K, MO, NY, SI); S. Mateis do Sul, S. Mateus, *Hatschbach 22284* (MO, NY, SI).

15. Verbena filicaulis Schauer, Prodr. 11: 549. 1847. TIPO: Brasil. 1835, *M. Lund 823* (lectotipo, designado por O'Leary (2006: 496), G no visto; isotipos, G foto F 7854!, SI!). Figura 9D–G.

Verbena pinnatisecta Schauer, Prodr. 11: 549. 1847. *Verbena filicaulis* var. *pinnatisecta* (Schauer) Moldenke, Phytologia 40: 261. 1978. TIPO: Brasil, *F. Sellow 4874* (lectotipo, designado por O'Leary (2006: 497), K no visto; isotipos, K foto SI!, SI!).

Verbena australis Moldenke, Phytologia 2: 419. 1948. *Verbena filicaulis* var. *australis* (Moldenke) Moldenke, Phytologia 40: 261. 1978. Syn. nov. TIPO: Brasil. Paraná: Jaguariahyva, *P. Dusén 13190* (holotipo, S no visto; isotipos, BAF!, NY!, S foto SI!, SI!).

Verbena regnelliana Moldenke, Phytologia 2: 425. 1948. Syn. nov. TIPO: Brasil. Minas Gerais: Caldas, in ripa uliginosa, Rio Verde, 20 Nov. 1867. *A. F. Regnell III 939* (holotipo, S no visto; isotipos, NY foto SI!, S foto SI!, SI!).

Hierbas de bajo porte, hasta de 40 cm de altura, de aspecto subgrácil, tallos herbáceos delgados erectos, tetrágonos, longitudinalmente estriados, ramificados desde la base, muchas veces ramas algo decumbentes en la base, luego erectas hacia el ápice o directamente procumbentes; los entrenudos basales breves, los apicales distanciados hasta de 10 cm; plantas glabras a ligeramente pubérulas o algo estrigosas. *Hojas* basales de lámina de (1.5–)2.2(–4) × (1–)2(–3) cm, morfología variable, bipinnatisecta o trilobada con lóbulo central entero a tripartido-multipartido, las hojas apicales disminuyendo en tamaño y en grado de división de la lámina llegando a enteras, angostas, base angostándose hacia un breve pecíolo de 0.2–0.5(–1) cm, ápice agudo a acuminado, margen multífido, subrevoluto, generalmente braquiblastos breves en axila de hojas basales, portadores de 1 ó 2 pares de hojas. *Florescencias* terminales, obovadas en antesis, de 0.5–2 × 0.5 cm, en fructificación alargadas hasta 4–7 cm. flores imbricadas en antesis y distanciadas 0.3–0.6 cm entre sí en fructificación, pedúnculos filiformes y largos de 3–7 cm; las florescencias se agrupan en paracladios trímeros, la florescencia principal pedunculada, las laterales superándola en la fructificación, sinflorescencia bracteosa de primer orden de ramificación; brácteas florales ovadas de ápice agudo, de (0.12–)0.2(–0.3) cm, pubérulas marginalmente a esparcidamente ciliadas. *Flores* sésiles, corola color lila o violáceo: cáliz de (0.25–)0.35(–0.43) cm, con 5 costillas escasamente tomentosas o glabras y 5 dientes breves triangulares de ápice mucronado; corola infundibuliforme, tubo corolino de (0.35–)0.45(–0.6) cm, pubescencia viloso lanosa en el interior de la garganta, subglabra en el exterior, apenas pubérula en la fauce, estambres insertos en la parte superior del tubo corolino. Clusas de 2–2.8 mm, dorso reticulado en el tercio superior, el resto estriado, cara comisural papiloso-verrucosa. Anatomía caulinar tipo A.

Distribución y ecología. Sur de Brasil en los estados de Minas Gerais, Paraná, Rio Grande do Sul,

Santa Catarina y São Paulo; Argentina en la provincia de Misiones. Probablemente habite en Paraguay. Se la encuentra en campos húmedos, márgenes de ríos y terrenos inundados.

Observaciones. *Verbena filicaulis* es la única especie dentro de la serie *Pachystachyae* que presenta hojas de lámina bipinnatisecta o trilobada, siendo éste el carácter que la diferencia, y posiblemente por el cual Schauer (1847) la incluyó en la serie *Leptostachyae* (= serie *Verbena*).

Material adicional examinado. ARGENTINA. **Misiones:** Dpto. Candelaria, Sta. Ana, *Rodriguez 570* (SI). BRASIL. **Minas Gerais:** entre Barbacena El sitio, *Glaziou 18391* (P, SI); s. loc., 1845, *Widgren s.n.* (P, SI). **Paraná:** Jaguariaiva, *P. Dusén 13053* (BAF); Tijucas do Sul, Rincão, *Hatschbach 40474* (CTES). **Rio Grande do Sul:** Itaimbezinho, San Francisco de Paula, *Batista 17710* (SI). **São Paulo:** s. loc., 1 Ene. 1912, *s. coll.* (SI). **Santa Catarina:** São Joaquin, Bom Jardim, *Reitz & Klein 7711* (SI); Mun. Agua Doce, *Smith & Klein 13390* (P, SI).

16. Verbena hirta Spreng., Syst. Veg. Ed. 16, 2: 749. 1825. TIPO: Brasil, *F. Sellow* (lectotipo, designado por O'Leary (2006: 496), K no visto; isotipo, K foto SI!).

Sufrútices, hasta de 80 cm de altura, tallos decumbentes y ramas ascendentes, en la base muchas veces tallos cilíndricos; pubescencia variable, generalmente plantas muy hirsutas, de aspecto canescente, hasta plantas poco pubescentes. *Hojas* de lámina entera, ovada, obovada, elíptica hasta ligeramente orbicular, sésiles a subpecioladas de base cuneada, los márgenes dentados o paucidentados hacia la porción distal de la lámina, con dientes agudos o crenas obtusas. *Florescencias* terminales, densas o laxas, de 1.5(–3) × 0.7(–0.9) cm, las laterales nunca superando la principal, agregadas en paracladios trímeros congestos o laxos, sinflorescencias frondosas de primer orden de ramificación, bráctea floral de longitud variable entre (0.15–)0.28(–0.5) cm, los márgenes pestañosos, subglabra a pubescente hacia el ápice. *Flores* sésiles de corola color lila o violáceo; cáliz de (0.2–)0.42(–0.6) cm, de igual longitud o mayor a la bráctea floral, subglabro entre costillas, híspido sobre las mismas, a veces pelos glandulares esparcidamente dispuestos, con 5 dientes triangulares evidentes o no, de ápice agudo; corola infundibuliforme, tubo corolino ca. 0.5–0.65 cm, poco mayor en longitud que el cáliz, pubescente en la garganta, fauce con pelos moniliformes; estambres insertos hacia la garganta del tubo corolino. Clusas de 2–2.5 mm, dorso reticulado en la mitad superior, el resto estriado, cara comisural papilosa. Anatomía caulinar tipo A.

Observaciones. *Verbena hirta* es un taxón que se reconoce por la pubescencia hirsuta a estrigosa, de densidad muy variable y las hojas de lámina ovada, obovada, elíptica hasta ligeramente orbicular, sésiles a subpecioladas de base cuneada. Se encuentran dos variedades de este taxón basadas en el tipo de florescencia, el porte y las dimensiones de las hojas y de las piezas florales.

CLAVE DE LAS VARIEDADES DE *VERBENA HIRTA*

16a. Florescencias fastigiadas, densifloras, sésiles, congestas en al ápice de ramas; bráctea floral de (0.2–)0.3(–0.5) cm . . . . . 16a. var. *hirta*

16b. Florescencias no fastigiadas, paucifloras hacia la base de las mismas, subpedunculadas, laxas en al ápice de ramas; bráctea floral de (0.15–)0.23(–0.3) cm . . . . . 16b. var. *gracilis*

16a. Verbena hirta var. **hirta** Spreng. Figura 10A–C.

Verbena paranensis Moldenke, Phytologia 6: 331. 1958. Syn. nov. TIPO: Brasil. Paraná: Guarapuava, 15 Nov. 1957, *G. Hatschbach 4214* (holotipo, TEX no visto; isotipos, TEX foto SI!, SI!).

Verbena hirta var. *dusenii* Moldenke, Phytologia 33: 480. 1976. Syn. nov. TIPO: Brasil. Paraná: Layo, 11 Dic. 1903, *P. Dusén s.n.* (holotipo, NY no visto; isotipos, NY foto SI!, SI!).

Tallo muchas veces leñoso en la base, hábito decumbente con ramas erectas ascendentes rígidas. Hojas de (1–)2–2.5(–4) × (0.6–)1–2(–3) cm, lámina ovada u obovada, generalmente sésiles a subsésiles, los márgenes paucidentados a crenados en la porción distal de la lámina con dos ó tres dientes agudos. Sinflorescencias fastigadas, capitado-congestas; brácteas florales de (0.2–)0.3(–0.5) cm, cáliz con 5 dientes triangulares evidentes de 0.6 mm.

Distribución y ecología. Argentina, en la provincia de Misiones y Brasil en los estados de Paraná, Rio Grande do Sul, Santa Catarina y São Paulo. Habita campo pedregoso, borde de pastizal pantanoso, o campos secos. Es frecuente en campos del estado de Paraná en Brasil, donde llega a ser una planta invasora.

Observaciones. *Verbena hirta* var. *hirta* se distingue de la variedad *gracilis* por su porte más erecto, sus hojas de mayores dimensiones y muchas veces híspidas de aspecto canescente.

Existe una marcada variación en cuanto a la pubescencia, habiendo ejemplares con pubescencia incana densamente vilosa e hirsuta, de aspecto canescente, hasta ejemplares escasamente pilosos. Los ejemplares *Smith & Klein 14936* (SI!), *Pereira 5481* (SI!), *Dombrowski 6359* y *6954* (SI!) son

altamente pubescente con ramas erectas con entrenudos muy breves, hojas sésiles pequeñas de 1–2 cm, de ápice agudo, adpresas al tallo, y florescencias breves terminales fastigiadas. Posiblemente la pilosidad sea una característica geográfica, dado que generalmente se encuentran en el estado de Paraná; hacia el sur las plantas son menos pubescentes y con hojas más grandes. Además se encuentran ejemplares (ej. *Smith & Klein 12118*, SI!; *Reitz 5210*, SI!) que no son cano pubescentes pero poseen hojas pequeñas, adpresas y entrenudos breves, lo cual confirma que esta forma no merece valor taxonómico.

Moldenke (1958: 331) al describir *Verbena paranensis* la distingue de *V. hirta* por tener "inflorescencia capitada y flores chicas". El análisis del ejemplar tipo y de material de herbario permitió comprobar que existe una marcada variación en los caracteres anteriormente señalados. Se encontraron ejemplares, con inflorescencias y flores semejantes al tipo de *V. paranensis* y el resto de la planta similar a *V. hirta* (ej. *Smith & Reitz 9162*, SI!). Consecuentemente los caracteres que definen *V. paranensis* no son relevantes ya que estarían dentro de las variaciones morfológicas que puede presentar *V. hirta*, y se incluye a *V. paranensis* bajo la sinonimia de *V. hirta*.

Moldenke (1976a: 480) diferencia *Verbena hirta* var. *dusenii* por las hojas más grandes y los entrenudos más elongados. Se han encontrado ejemplares (*Capell 1*, SI!; *Smith & Klein 13733*, SI!; *Dusén 13239*, SI!; *Reitz & Klein 13885*, SI!) que presentan entrenudos breves y entrenudos largos en un mismo ejemplar, con hojas de dimensiones grandes y pequeñas. Por lo tanto estos caracteres son taxonómicamente irrelevantes para definir la variedad, siendo *V. hirta* un taxón polimórfico para los mismos.

Material adicional examinado. ARGENTINA. **Misiones:** Bdo. De Irigoyen, *Eskucke 5249* (SI). BRASIL. **Paraná:** Correg o da Anta, *Pedersen 10952* (CTES, SI); s. loc., 22 Oct. 1911, *Dusén 13239* (SI); Curitiba, rio Atuba, *Hatschbach 25608* (SI); Vila Velha, *Dombrowski 9464* (SI); Campo Largo, S. Luiz do Puruña, *Dombrowski 6359* (SI); Campo Largo, S. Luiz do Puruña, *Dombrowski 6954* (SI); Campo Largo, rio Papagaio, *Pereira 5481* (SI); Palmeira, Papagaio, *Smith & Klein 14936* (SI). **Rio Grande do Sul:** Julio de Castilhos, BR 158, *Pedersen 11485* (SI). **Santa Catarina:** Agua Doce, rio Paseira, *Krapovickas & Cristóbal 33676* (CTES); Abelardo Luz, 9 km N Abelardo Luz, *Smith & Klein 13885* (SI); Campo Ere, 6 km W Campo Ere, *Smith & Klein 13733* (SI); 13 km NW Mafra, *Smith & Klein 12118* (SI); Mafra, *Reitz 5210* (SI); Joacaba, *Smith & Reitz 9162* (SI). **São Paulo:** Jordan, *Capell 5* (SI); Campos de Jordán, *Capell 1* (SI).

16b. Verbena hirta var. **gracilis** Dusén, Arq. Mus. Nac. Rio de Janeiro 13: 36. 1903. TIPO: Brasil. Serra do Itatiaia, Pedra Assentada, 15 Mayo 1902, *P. Dusén 232* (holotipo, S no visto; isotipo, S foto SI!). Figura 10D, E.

Verbena strigosa Cham., Linnaea 7: 256. 1832. Syn. nov. TIPO: Brasil. *F. Sellow* (lectotipo, designado por O'Leary (2006: 498), K no visto; isotipos, BR foto SI!, K foto SI!, P 00289960 foto SI!, SI!).

Verbena reitzii var. *castrensis* Moldenke, Phytologia 13: 259. 1966. Syn. nov. TIPO: Brasil. Paraná: Castro, Carambei, 15 Nov. 1965, *L. B. Smith, R. Klein & G. Hatschbach 14526* (holotipo, TEX no visto; isotipos, NY foto SI!, SI!, TEX foto SI!, US foto SI!) Figura 10D.

Tallo herbáceo, hábito decumbente con ramas erectas no rígidas. Hojas de (0.5–)1.5–2(–3) × (0.4–)0.6–1(–2) cm, lámina elíptica a ligeramente obovada u orbicular, subpecioladas, los márgenes paucidentados con 2–3 dientes a cada lado o crenas redondeadas. Florescencias laxas, paucifloras hacia la base; brácteas florales de (0.15–)0.23(–0.3) cm, cáliz con 5 dientes evidentes o cortos, glabro entre costillas.

Distribución y ecología. *Verbena hirta* var. *gracilis* es simpátrica con la variedad tipo, crece en los estados del sur de Brasil hasta São Paulo, siendo bastante frecuente en el estado de Paraná. Es una hierba que habita campos secos y limpios.

Observaciones. *Verbena hirta* var. *gracilis* se distingue de la variedad tipo por el porte grácil, la menor pubescencia, las hojas subpecioladas y las florescencias menos robustas con pedúnculos más largos. Muchas veces predomina la pubescencia estrigosa, con pelos adpresos, encontrándose en la variedad tipo generalmente pelos híspido-hirsutos.

Verbena strigosa fue caracterizada por ser plantas de bajo porte, con hojas pequeñas hasta de 3 cm y florescencias paucifloras laxas (Chamisso, 1832). El análisis del material tipo de ambos taxones, junto con el material de herbario (ej. *Burkart 25168*, SI!; *Sobral 1251*, SI!) permitió determinar que la variedad *gracilis* no se puede diferenciar de *V. strigosa*, y ambos taxones son tratados aquí como variedad *gracilis*.

La descripción original de *Verbena reitzii* var. *castrensis* (Moldenke, 1966: 259) y el análisis del material tipo permitió determinar que no existen diferencias morfológicas con *V. hirta* var. *gracilis*.

Material adicional examinado. BRASIL. **Paraná:** Curitiba, Ponta Grossa, *Dombrowski 2201* (SI); Guarapuava, *Reitz & Klein 17616* (SI); Laranjeiras do Sul, *Hatschbach 35191* (LP); Castro, *Hatschbach 20073* (SI). **Rio Grande do Sul:** Itaimbezinho, Cambará do sul, *Sobral 1251* (SI); S. Francisco de Paula, *Burkart 25168* (SI). **Santa Catarina:** Curitibanos, Campos Novos, *Smith & Klein 11107* (SI); Lajes, *Smith & Reitz 10024* (SI); Mun. São Joaquin, serra do Oratorio, *Lourteig 2143* (SI).

17. Verbena lobata Vell., Fl. Flumin. 18: 1825; Icon. 1: tab. 43. 1827. TIPO: Vellozo, 1827. Icon. 1: tab. 43 (lectotipo, aquí designado).

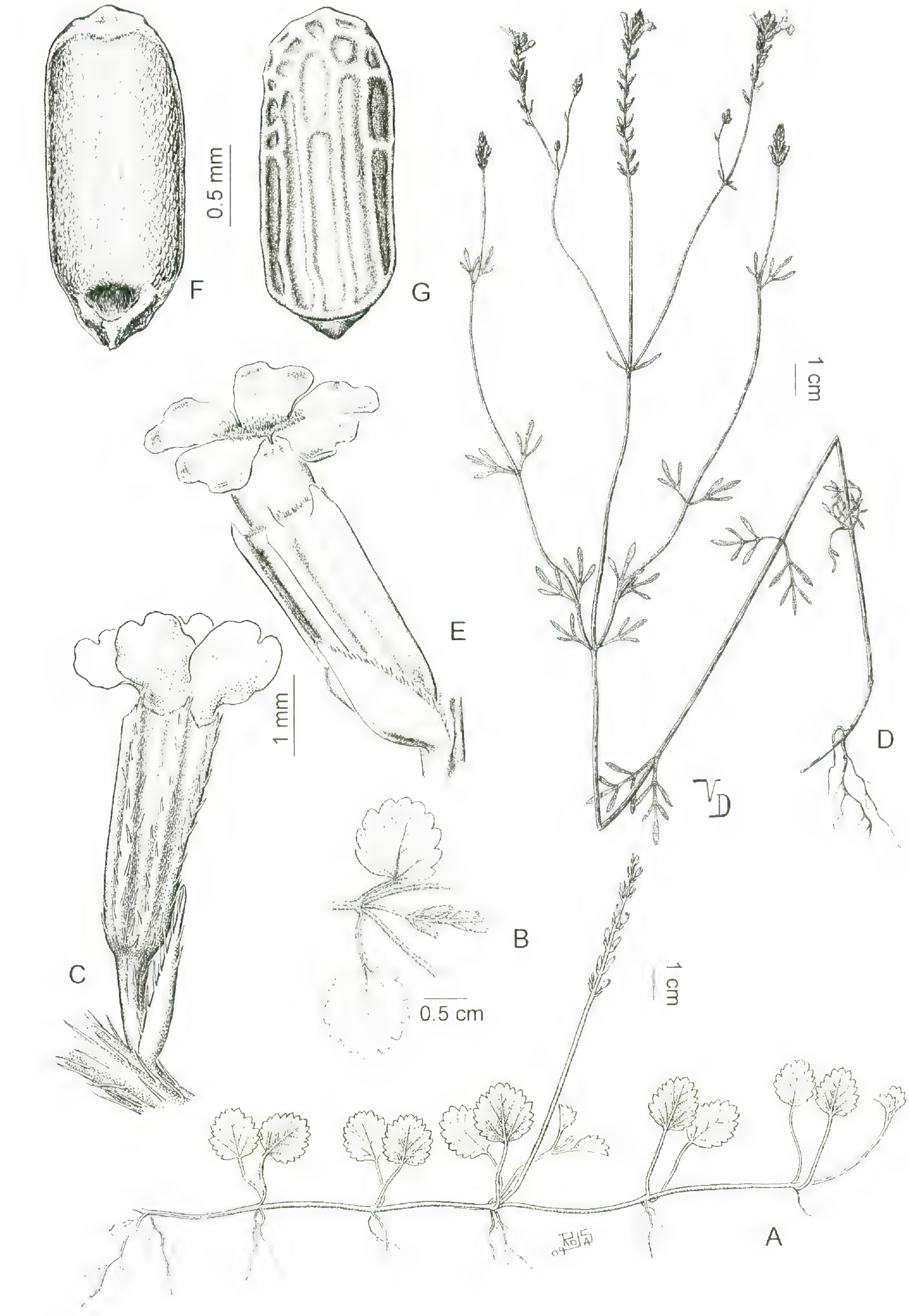


Figura 9. A–C. *Verbena caniuensis*. —A. Aspecto general de la planta con inflorescencia. —B. Detalle nudo, donde se aprecia que la florescencia es terminal y las yemas axilares desarrollan generando aspecto rastrero. —C. Flor. D–G. *Verbena filicaulis*. —D. Aspecto general de la planta con inflorescencia. -E. Flor. —F. Clusa, vista ventral. —G. Clusa, vista dorsal. A–C: de *Hatschbach 20177* (SI); D–G: de *Rodriguez 570* (SI).

Hierbas perennes de aspecto grácil, de 0.3–0.6(–1.6) m de altura, ramas y tallos erectos, ramificaciones abiertas, pubescencia variable, subglabras, estrigosas, híspidas o hirsutas con pelos hasta de 3 mm, con o sin pelos glandulares. *Hojas* de lámina entera, de 3–6 × 2–4 cm, ovadas, triangulares, a veces ligeramente trilobadas hacia la base, ápice agudo, base subtruncada o subredondeada, pecíolo de 0.5–1 cm, márgenes crenado-serrados a lo largo de toda la lámina, dientes irregulares acuminados, prominente venación en cara abaxial, marcada pubescencia sobre las venas. *Florescencias* terminales, cilíndricas, poco densas o laxas, breves y anchas de 1–2 × 0.7–0.8 cm, reunidas en paracladios laxos, sinflorescencia paniculosa amplia de aspecto piramidal en fructificación, más compacta en antesis, bracteosa con ramificaciones de segundo orden; brácteas florales breves, de 0.2–0.4 cm, ovadas, de ápice agudo, siempre menores al cáliz en longitud. *Flores* sésiles, corola violeta, rosa a veces azul o purpúrea; cáliz de 0.4–0.6 cm, con 5 dientes triangulares de ápice agudo de 0.5–1 mm, conniventes en fruto, no contortos; corola infundibuliforme, tubo corolino apenas exerto del cáliz, de 0.5–0.7 cm, pubescencia vilosa externa, pelos moniliformes en la garganta; presencia de receptáculo floral desarrollado, estambres superiores con o sin apéndices glandulares; estilo de 0.4 cm. Clusas de 2 mm, dorso reticulado en el tercio superior, el resto estriado, cara comisural papilosa, base del mericarpio no angostada y con repliege basal de la pared comisural. Anatomía caulinar tipo A.

CLAVE PARA LAS VARIEDADES DE *VERBENA LOBATA*

17a. Plantas con pubescencia híspida o hirsuta, con pelos hasta de 3 mm, con pelos glandulares; sinflorescencia muy desarrollada . . . 17a. var. *lobata*

17b. Plantas subglabras, piezas florales y raquis con pelos breves, adpresos, nunca pelos glandulares; sinflorescencia poco desarrollada . . 17b. var. *glabrata*

17a. Verbena lobata var. **lobata** Vell.

Verbena buchnera Vell., Fl. Flumin. 17: 1825; Icon. 1: tab. 42. 1827 [1831]. TIPO: Vellozo, 1827, Icon. 1: tab. 42 (lectotipo, aquí designado).

Verbena lobata var. *hirsuta* Moldenke, Phytologia 2: 423. 1948. Syn. nov. TIPO: Brasil. Rio Grande do Sul: Pinhal prope Santa Maria, 27 Nov. 1902, *G. O. A. Malme 1260* (holotipo, S no visto; isotipos, NY foto SI!, S foto SI!).

La inflorescencia es amplia, conformada por paracladios trímeros o muchas veces tetrámeros; pubescencia híspida a hirsuta de densidad variable, piezas florales con pubescencia híspida con pelos glandulares.

Distribución y ecología. Noreste de Argentina, sur de Brasil y este de Paraguay. Habita en campos.

Observaciones. Se desconoce donde están depositados los ejemplares de Vellozo (Stafleu & Cowan, 1986). No se encontró ningún ejemplar que pudiera ser material original de *Verbena lobata* Vell. ni de *V. buchnera* Vell.; por lo tanto se designó lectotipo la lámina de *Flora Fluminensis* citada en el protólogo de ambos taxones (Art. 9.10; Greuter et al., 2000).

Chamisso (1832: 255) menciona *Verbena corymbosa* de Ruiz y Pavón y cita un ejemplar coleccionado por *Sellow* en Brasil. Este ejemplar se encuentra en herb. K y corresponde a *V. lobata* por lo cual el nombre *V. corymbosa* aplicado por Chamisso es una mala determinación. *Verbena corymbosa* sería una *Glandularia* (véase Taxones Dudosos).

En *Verbena lobata* es frecuente observar cuatro florescencias surgiendo de un mismo nudo, lo cual da un aspecto piramidal a la inflorescencia. A su vez, también se observó que las florescencias más alejadas de la terminal suelen ser paucifloras, hasta de 2 flores por florescencia.

Moldenke (1948a) diferencia *Verbena lobata* var. *hirsuta* por la presencia de pubescencia densa hirsuta con pelos blancos flavescentes. Sin embargo *V. lobata* var. *lobata* posee pubescencia muy variable, con pelos híspido o hirsutos bastante evidentes y este carácter no permite diferenciar ambos taxones. Moldenke (1948a) en el protólogo cita como paratipos de la var. *hirsuta* los ejemplares *Rambo 53791* (SI!), *Sehnem 3497* (SI!), *Smith & Klein 8105* (SI!); el estudio de los mismos no permite diferenciarlos por su pubescencia de otros ejemplares determinados como la variedad tipo.

Material adicional examinado. ARGENTINA. **Misiones:** San Pedro, 4 km NE San Pedro, *Correa 5482* (SI); San Pedro, arroyo Fortaleza, *Gamerro 38* (SI); Gral. M. Belgrano, Bdo. De Irigoyen, Pepirí Guazú, *Morrone et al. 1344* (SI). BRASIL. **Paraná:** Curitiba, Barigui, *Hatschbach 52324* (SI); Guarapuava, Guara, *Hatschbach 19753* (SI); Piraquara, Mananciais da Serra, *Dombrowski 2765* (SI); San José dos Pinhaes, Lagoinha, *Hatschbach 2873* (SI). **Rio Grande do Sul:** Granja Sodol, Giruá, *Hagelund 4741* (SI); Montenegro, *Sehnem 4981* (SI); L. Pinhal, *Sehnem 3497* (SI); S. F. De Paula, Vila Oliva, *Rambo 30942* (SI); serra da Rocinha, *Rambo 53791* (SI). **Santa Catarina:** Araranguá, Sanga D'Anta, capoeira, *Reitz c1215* (SI); Santa Cecilia, Beira de capao, *Reitz & Klein 13511* (SI); betw. Palmeiras & Lajes, *Smith & Klein 8105* (SI); Rio dos Patos, Lebon Regis, *Reitz & Klein 13873* (SI); S. Joaquim, B. Jardim, serra do Oratorio, *Reitz & Klein 7691* (SI). PARAGUAY. **Alto Paraná:** in regione fluminis, *Fiebrig 5884* (SI).

17b. Verbena lobata var. **glabrata** Moldenke, Phytologia 3: 118. 1949. TIPO: Brasil. Rio Grande do Sul: S. Fco. de Paula, 14 Ene. 1937,

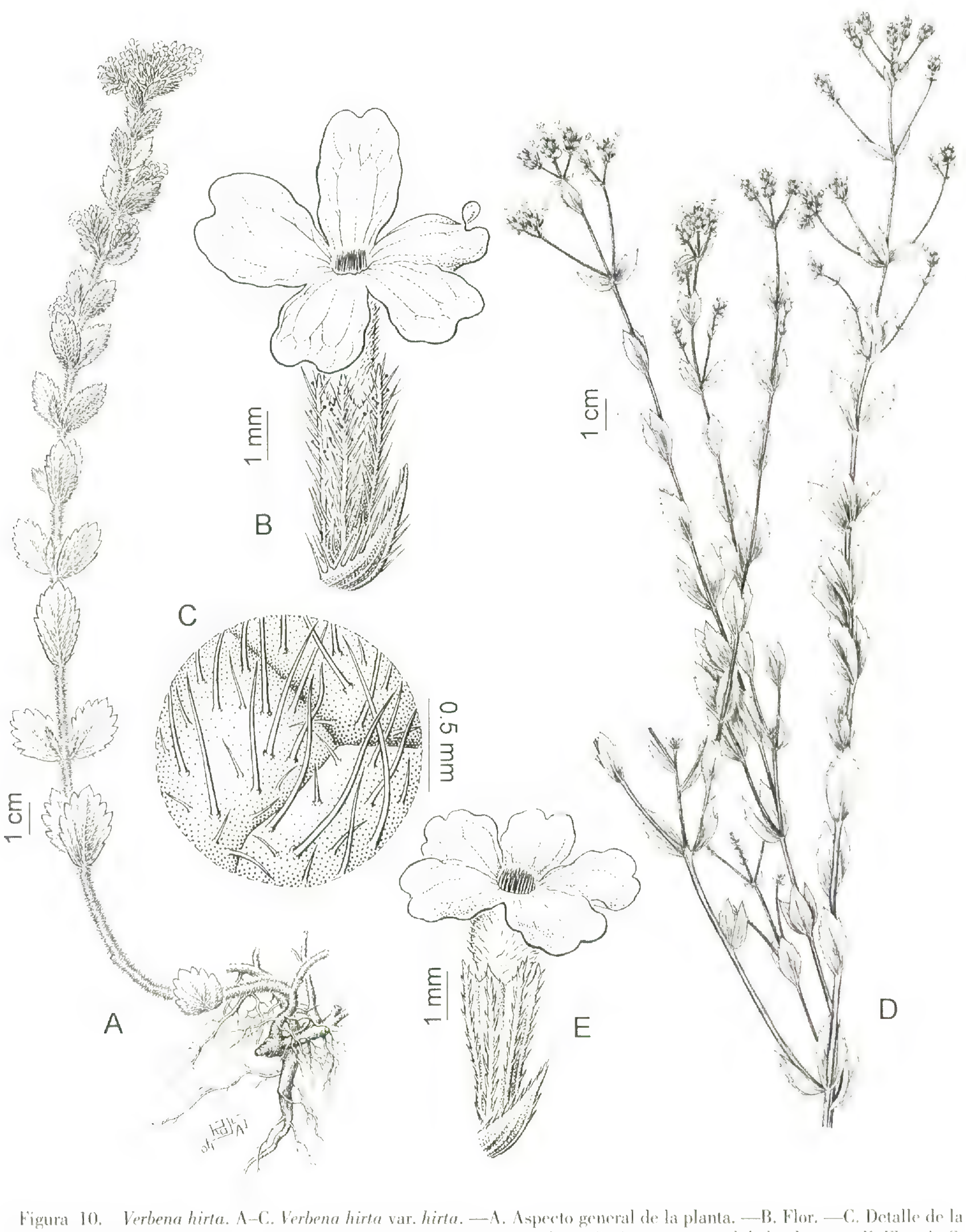


Figura 10. *Verbena hirta*. A–C. *Verbena hirta* var. *hirta*. —A. Aspecto general de la planta. —B. Flor. —C. Detalle de la pubescencia de la cara abaxial de la hoja. D, E. *Verbena hirta* var. *gracilis*. —D. Aspecto general de la planta. —E. Flor. A–C: de *Pedersen 10952* (SI); D: de *Smith et al. 14526* (SI), espécimen tipo de *V. reitzii* var. *castrensis*; E: de *Burkart 25168* (SI).

B. Rambo 2816 (holotipo, NY no visto; isotipos, NY foto SI!, SI!).

La inflorescencia es poco desarrollada, generalmente conformada por un paracladio trímero; pubescencia escasa, estrigosa con pelos breves, adpresos, sin pelos glandulares en las piezas florales.

Distribución y ecología. La variedad *glabrata* posee una distribución más restringida que la

variedad tipo, hallándose únicamente en los estados del sur de Brasil.

Material adicional examinado. BRASIL. **Rio de Janeiro:** Serra da Bocaina, *Duarte 7693* (SI); Serra dos Orgãos, *Capell 8* (SI). **Santa Catarina:** Campo Alegre, Morro do Iquererim, *Reitz & Klein 5226* (SI); Morro do Iquererim, campo Alegre, *Reitz & Klein 5171* (SI); Serra da Boa Vista, São José, *Reitz 5412* (SI).

18. Verbena subpetiolata N. O'Leary, nom. nov.

Nombre reemplazado: *Verbena lobata* var. *sessilis* Moldenke, Phytologia 4: 292. 1953, non *Verbena sessilis* (Cham.) Kuntze, Revis. Gen. Pl. 3(2): 257. 1898. TIPO: Brasil. Paraná: S. José dos Pinhaes, Varzea, 2 Dic. 1952, *G. Hatschbach 2876* (holotipo, NY no visto; isotipos, NY foto SI!, SI!).

Hierbas perennes, subrastreras, ramas y tallos decumbentes en la base, erectos hacia el ápice, hasta de 30 cm alt., pubescencia variable híspida o hirsuta, pelos hasta de 2 mm, con pelos glandulares. *Hojas* de lámina entera, de 1–2(3) × 0.5–1(2) cm, ovadas, de ápice subobtuso, base subtruncada a redondeada, sésiles a subsésiles, márgenes inciso-serrados a lo largo de toda la lámina, a veces subcrenados, marcada pubescencia sobre las venas. *Florescencias* terminales, capituliformes en antesis, cilíndricas en fructificación, laxas hacia la base, breves y anchas de 2–4 × 0.5–0.6 cm, reunidas en paracladios trímeros o solitarias, sinflorescencia frondosa de primer orden de ramificación; brácteas florales breves, de 0.2–0.4 cm, ovadas, de ápice agudo, siempre menores al cáliz en longitud. *Flores* sésiles, corola violeta, rosa a veces azul o purpúrea; cáliz de 0.4–0.5 cm, con 5 dientes triangulares, de 0.5–1 mm, de ápice agudo, conniventes en el fruto, no contortos, pubescencia híspido-glandular; corola infundibuliforme, tubo corolino apenas exerto del cáliz, de 0.5–0.7 cm, pubescencia vilosa externa, pelos moniliformes en la garganta; presencia de receptáculo floral desarrollado; estambres superiores con apéndices glandulares, casi protruyendo de la corola; estilo de 0.4 cm. Clusas de 2 mm, dorso reticulado en el tercio superior, el resto estriado, cara comisural papilosa, base del mericarpio no angostada y con repliege basal de la pared comisural. Transcorte caulinar tipo A.

Distribución y ecología. Habita en campos del sur de Brasil, en los estados de Santa Catarina y Paraná.

Observaciones. El análisis del ejemplar tipo de *Verbena lobata* var. *sessilis* permitió observar que se trata de un taxón diferente de *V. lobata*, por poseer las hojas sésiles a subsésiles y de ápice subobtuso y sinflorescencia frondosa formada por paracladios trímeros o solitarios, de primer orden de ramificación, siendo en *V. lobata* las hojas pecioladas y de ápice agudo y la sinflorescencia bracteosa con paracladios de primer y segundo orden de ramificación.

Material adicional examinado. BRASIL. **Paraná:** Porto Vitoria, *Hatschbach 14883* (SI); União da Vitoria, *Koezicki 26* (SI). **Santa Catarina:** Araranguá, Taimbesinho, *Reitz 2170* (SI); Bom Retiro, Campo dos Padres, *Smith & Reitz 10341* (SI); Matos Costa, *Reitz & Klein 13710* (SI); Porto União, *Reitz & Klein 13611* (SI); Valoes, *Reitz & Klein 11551* (SI).

TAXONES DUDOSOS

Verbena concepcionis Moldenke, Phytologia 2: 236. 1947. TIPO: Chile. Concepción: 1789–1791, *L. Née 57* (holotipo, MA no visto; isotipos, MA foto SI!, NY foto SI!; SI!).

El material del cual se dispone resulta insuficiente para resolver este taxón ya que no se ha encontrado ninguna colección y únicamente se cuenta con fotos del ejemplar tipo y un fragmento infértil a partir de este ejemplar.

Verbena corymbosa Ruiz & Pav., Fl. Peruv. 1, 22, t. 33. 1798. TIPO: Chile. Concepción: *Ruiz & Pavon* (holotipo, MA no visto; isotipo, MA foto SI!).

Este taxón comparte ciertos caracteres con el género *Glandularia*, como la flor de tubo corolino largo y estilo largo, el cáliz fructífero con dientes contortos y el corte transversal del tallo con parénquima clorofiliano de disposición contínua (Botta, 1989). Sin embargo la inflorescencia es típica de *Verbena*, no habiendo glandularias que presenten las florescencias reunidas en paracladios de aspecto corimboso-compacto como en este caso (Peralta, comun. pers.). Por el momento se coloca como taxón dudoso a la espera de más resultados que permitan definir su posición.

Verbena trifida var. *deserticola* Moldenke, Phytologia 3: 281. 1950. TIPO: Chile. Antofagasta: Quebrada la Chimba, Sep. 1940, *Barros 8010* (holotipo, NY no visto; isotipo, SI!).

No se ha podido tener acceso a la foto del ejemplar tipo de NY. Michael Nee (comun. pers.) dice que el material tipo estaría en préstamo; el fragmento del cual se dispone en SI resulta insuficiente para decidir sobre este taxón.

Verbena valerianoides Kunth, Nov. Gen. Sp. (quarto ed.) 2: 277. 1818. TIPO: Colombia. "In frigidis Andium prope Sta. Fé de Bogotá et Chipo...", s.d., *Humboldt & Bonpland s.n.* (holotipo, P no visto; isotipo, P foto SI!).

Este taxón se asemeja a *Verbena lindbergii* Moldenke, una especie que crece en el noroeste de Argentina, en la provincia de Misiones y en los

estados del sur de Brasil hasta Paraná. Sin embargo, I. *valerianoides* fue fundada por Kunth (1818) sobre un ejemplar colombiano, y en la actualidad existen muy pocos ejemplares coleccionados, todos ellos de Colombia. Hasta que no se puedan coleccionar nuevos ejemplares este taxón será considerado como dudoso.

TAXONES INVÁLIDOS

Patya Neck., Elem. Bot. 1: 296. 1790, nom. inval.
Stylodon Raf., Neogenyton: 2. 1825, nom. inval.
Shuttleworthia Meisn., Pl. Vasc. Gen. 1: 290. 1840, nom. inval.

Los tres son nombres inválidos, citados por algunos autores (Perry, 1933: 251; Munir, 2002: 25; Atkins, 2004) como sinónimos del género *Verbena*.

Los nombres de Rojas (1897: 76, 173) *Verbena albicans* y *V. nigricans*, son nomina nuda según Krapovickas (1970: 256), y por lo tanto son nombres inválidos.

Los nombres de Larrañaga (1923) *Verbena repens* y *V. parviflora*, y el nombre *V. venosa* var. *parviflora* Thell. & Zimmerm. ex Hegi (1927) son nombres inválidos según Art. 32.1 de Greuter et al. (2000).

Perry (1933) menciona *Verbena integrifolia* Sessé & Moç. (Sessé & Moçiño, 1887: 6) cuestionando su identidad; Moldenke (1964a: 372) la considera una especie válida a pesar de no haber visto el tipo; Rzedowski y Rzedowski (2002) la coloca bajo la sinonimia de *V. litoralis* pero con un signo de interrogación. McVaugh (1998), en su trabajo sobre las plantas de Sessé y Moçiño, menciona que el tipo de este taxón no fue hallado; por lo tanto se trata de un nombre inválido.

Moldenke (1964d: 84) menciona *Verbena rugosa* Mill. (Miller, 1798) como especie dudosa; no se ha localizado ningún ejemplar tipo; López-Palacios (1977) y Munir (2002) mencionan *V. litoralis* var. *glabrior* Benth. (1846: 153) bajo la sinonimia de *V. litoralis* sin haber visto el tipo; ambos son nombres inválidos.

Verbena rigida f. *alba* (Trivetts) Moldenke (1977b), *V. rigida* var. *lilacina* (Benary & Bodger ex G. Harrow) Moldenke (1955) y *V. rigida* f. *lilacina* (Benary & Bodger ex G. Harrow) Moldenke (1977b), son nombres inválidos según Art. 32.1 de Greuter et al. (2000).

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Apéndice 1. Lista de especies y variedades aceptadas de *Verbena* serie *Pachystachyae*.

1. *Verbena alata* Otto ex Sweet
2. *Verbena bonariensis* L. var. *conglomerata* Briq.
3. *Verbena bonariensis* var. *bonariensis*
4. *Verbena caniuensis* Moldenke
5. *Verbena ephedroides* Cham.
6. *Verbena filicaulis* Schauer
7. *Verbena glabrata* Kunth
8. *Verbena hirta* Spreng. var. *gracilis* Dusén
9. *Verbena hirta* var. *hirta*
10. *Verbena hispida* Ruiz & Pav. var. *obovata* (Moldenke) N. O'Leary
11. *Verbena hispida* var. *hispida*
12. *Verbena intercedens* Briq.
13. *Verbena intermedia* Gillies & Hook. ex Hook.
14. *Verbena litoralis* Kunth var. *brevibracteata* (Kuntze) N. O'Leary
15. *Verbena litoralis* var. *litoralis*
16. *Verbena lindbergii* Moldenke
17. *Verbena lobata* Vell. var. *glabrata* Moldenke
18. *Verbena lobata* var. *lobata*
19. *Verbena montevidensis* Spreng.
20. *Verbena ovata* Cham.
21. *Verbena rigida* Spreng.
22. *Verbena sagittalis* Cham.
23. *Verbena subpetiolata* N. O'Leary

Apéndice 2. Índice de colecciones. Cada especímen es citado por el nombre del primer colector en el caso en que más de dos colectores hayan participado de la colección; si fueron dos colectores se citan ambos. En el caso de no poseer número de colección se indica el número del herbario en que se encuentra depositado o en su defecto sólo el herbario. Se indica entre paréntesis el número de orden del taxón a que se corresponde (véase Apéndice 1).

Abbiatti D. & Claps 707 (15); **Acevedo Rdgz. P.** 2980 (15); **Aguilar R. M.** 803 (15); **Aguirre E.** 14 (3), 447 (11); **Ahumada** 450 (15), 1948 (14), 2825 (3), 2896 (15), 2957 (2), 3006 (2), 3096 (3), 6232 (11); Ahumada et al. 765 (14), 2337 (3); Ahumada & Eskucke 2963 (21), 2970 (16), 3218 (21), 3340 (16), 3490 (21); Ahumada & Rotman 4410 (15); Ahumada & Schinini 4061 (2); **Alvarez R.** 183 (19), 311 (15); **Amorín J.** 132 (15); **Ance** 78 (15); **Anderson D. L.** 1830 (15); Anderson et al. 1645 (13); **Anetto L.** 9886 (13); **Arbo M. M.** 303 (21); Arbo & Schinini 2454 (13); Arbo et al. 911 (3), 1245 (14), 1302 (3), 1905 (21), 7734 (21), 8635 (12). **Arenas P.** 86 (15), 100 (14), 207 (14), 949 (14), 1273 (15), 2369 (11), 2928 (15); Arenas et al. 913 (11), 915 (15); **Argañarás** 52 (11); **Ariza Espinar L.** 2402 (15); **Astegiano M.** 8 (19). **Bacigalupo N.** 22827 (13); Bacigalupo et al. 888 (2); **Baer G.** 102 (11); **Báez F.** 29 (13), 454 (21); **Bastián** 492 (10); **Basualdo I.** 141 (14), 720 (19), 843 (19), 2070 (3), 2114 (15), 6028 (15), 6777 (14), 7176 (3), s.n. 1984 (21); **Batista W.** 47710 (6); **Bazzi R.** 229 (21); **Beck S. G.** 2268 (11), 2445 (11), 2751 (10), 3737 (15), 3905 (10), 4158 (11), 4575 (10), 7402 (10), 8314 (15), 8834 (11), 12001 (15), 14631 (11), 19814 (10); Beck et al. 12454 (11), 22029 (11); **Belgrano, M. J.** et al. 277 (3), 280 (15), 297 (15), 420 (19), 421 (19); **Bermdt M.** 5160 (13); **Biganzoli F.** et al. 254 (19), 298 (19), 562 (19), 909 (5), 1727 (13); **Biraben M.** 31 (3), 5220 (21), 5338 (15), 5409 (19), 5420 (13); **Biurrun F.** 7718 (13); Biurrum & Pagliari 6408 (14); Biurrum et al. 2561 (13), 2543 (13) 2543 (13), 6248 (13); **Blanchourd** 2217 (21), 2482 (13); **Boelcke O.** 403 (14), 2138 (3), 3921 (14), 5093 (19), 6753 (3), 8501 (3), 11827 (13); **Boffa P.** SI 25870 (3); **Bordini** 4 (19); **Bordón** et al. 2561 (15); **Botta S.** 147 (15); Botta & Miconi 361 (11), 386 (11), 665 (13); Botta et al. 254 (13); **Botten** 426 (15); **Bottino O.** 183 (19); **Breschia** et Marchesi 4157 (8); **Bridarolli A.** 625 (13), 2546 (20), 3024 (20); **Brollo** 92 (13), 78 (15); **Bruch** 2875 (15), 2724 (11); **Buchtien O.** 196 (11), 976 (15), s.n. 1911 (15), s.n. 1895 (15), s.n. SI 3401 (15); s.n. SI 3402 (15); nro. Gay 22 (14); **Bueno O.** 1109 (19), 2707 (15); **Burkart A.** 376 (21), 915 (2), 950 (3), 1430 (21), 2145 (15), 3272 (3), 3367 (19), 3426 (18); 4268 (13), 6671 (3), 6678 (13), 7493 (13), 7890 (21), 7949 (21), 8053 (13), 8258 (3), 8278 (2), 8306 (15), 8736 (15), 8799 (15), 9304 (15), 10062 (19), 10061 (15), 10063 (13), 10405 (13), 10411 (11), 11008 (15), 11060 (13), 12825 (3), 13205 (13), 14067 (21), 14426 (20), 15773 (21), 16016 (15), 16170 (15), 16815 (15), 17069 (15), 17788 (19), 17844 (13), 18506 (15), 19557 (13), 20520 (3), 20622 (19), 21554 (2), 22283 (13), 22373 (13), 22705 (13), 25163 (1), 25164 (16), 25167 (6), 25168 (8), 25175 (21), 25176 (15), 26036 (13), 27701 (19), 27764 (2); Burkart & Bacigalupo 21367 (15), 21370 (13); Burkart & Crespo 23097 (2), 23100 (13), 23101 (19), 23102 (19); Burkart & Gamerro 21888 (3); Burkart & Troncoso 24229 (19), 27352 (14), 27357 (2), 28060 (21); Burkart et al. 14273 (11), 25110 (13), 27058 (3), 30108 (15), 30112 (21), 30597 (11), 30601 (11), 31051 (14); **Burns** BAB 6661 (21). **Cabral E. L.** & Molina 798 (15); Cabral et al. 323 (22), 386 (22); **Cabrera A.** 88 (3), 1710 (15), 2108 (15), 4326 (13), 4885 (3), 4900 (3), 5148 (3), 5200 (14), 5504 (13), 5630 (13), 5660 (13), 6925 (13), 8198 (15), 8496 (3), 8569 (15), 9840 (13), 10161 (19), 27646 (19), 27759 (21), 28092 (13), 28323 (21), 28473 (21), 28628 (19), 31920 (15), 32103 (15), 33946 (15), 34404 (15); Cabrera & Fabris 15982 (14); Cabrera & Kiesling 20382 (15); Cabrera & Troncoso 29279 (21); Cabrera & Zuloaga 32353 (21), 32420 (13); Cabrera et al. 13747 (15), 14657 (15), 17983 (15), 21335 (3), 24333 (11), 26133 (15), 27655 (13), 27910 (15), 28415 (13), 28488 (21), 28558 (21), 28616 (2), 28683 (21), 28978 (13), 29151 (19), 29755 (11), 29847 (15), 29873 (15), 29945 (11), 31617 (11), 32155 (15), 33929 (15), 34519 (15), 34553 (14), 34636 (15), 34771 (15); **Cáceres E.** 96 (11), 1823 (15); **Calviño C.** 623 (19); **Callejas R.** et al. 2288 (15); **Cano A.** 2705 (5), 8335 (11); **Capell** s.n. (9), 1 (9), 3 (9), 4 (15), 5 (9) 6 (9), 8 (17); **Capurro R.** BA 55838 (14); **Carminati** 23 (3); **Carnevale** 82 (13); **Carnevali R.** 400 (3), 2622 (13), 2879 (3), 2987 (3), 3690 (13); **Caro J.** 1603 (19), 1643 (13), 1758 (13), 2150 (3), 2702 (3), 2705 (5), 2981 (13), 3230 (11), 4775 (2), 5000 (10); Caro & Cámara Hernandez 548 (21), 571 (15); Caro J. & Cocucci 3415 (15). **Castañeda Vega** 59 (15); **Castellanos A.** BA nro. 24/1931 (3), BA nro. 25/933 (3), BA nro. 25/930 (15), BA nro. 27/208 (13), BA nro. 27/2031 (15), BA nro. 28/324 (15), BA nro. 31/1285 (19), BA nro. 31/1277(15), BA nro. 31/1281 (2), SI nro. 3824 (11), SI nro. 3827 (13), s.n. 1924 (3) 154 (19), 434 (3); 706 (13), 10829 (13), 10833 (3), 19613 (15), 23611 (2), 46956 (11), 51170 (11); **Castro M.** BAB 52324 (2); **Castro T.** 125 (15); **Cazzaniga** 4061 (19), 5035 (15), 5343 (14), 5562 (14); **Ceballos** et al. 105 (11); **Chalukian S.** 416 (15); **Charpin A.** & Eskucke AC20123 (3), 20407 (15); **Cialdella A.** et al. 77 (3), 81 (15); **Claren F.** 11558 (11); **Clos E.** 4126 (3), 4150 (3); **Cocucci A.** et al. 3028 (2), 3142 (15), 3287 (10); **Cordini R.** 76 (11); **Corette** & Ruiz Leal 3891 (3); **Cordo**-Ferrer s.n. (11); Cordo 87-A 31 (15); **Correa** 5482

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(10); **Di Giacomo A.** 48 (3), 448 (21); **Di Iorio O.** 07 (15); **Dombrowski L.** 139 (8), 1796 (19), 2006 (9), 2201 (9), 2272 (16), 2705 (18), 2765 (18), 2958 (19), 3066 (18), 3139 (19), 3227 (9), 3731 (18), 3796 (9), 3954 (16), 4228 (18), 4307 (6), 4439 (3), 4852 (15), 5686 (15), 6359 (9), 6680 (21), 6704 (19), 6789 (9), 6931 (9), 6954 (9), 7735 (16), 9103 (3), 9464 (9), 11078 (8); **Doria** 2527 (19); **Duarte** 7693 (17); **Dusén P.** 13053 (6), 13239 (9), 13329 (21); **Duvango** 56 (11). **Elisetch M.** 268 (11); **Emrich K.** 89 (15), 37454 (13); **Escobar A.** X-200 (11); **Eskueke U.** 5249 (9). **FLSP** 2191 (7); **Fabris H.** 2647 (19), 3434 (15), 5118 (3), 5233 (11), 5308 (15), 6814 (13), 6906 (11), 8280 (15); Fabris & J. Hunziker 7378 (13); Fabris & Pérez Moreau 6702 (13); **Faggi A.** 19003 (14), Faggi & Cagnoni BA 78919 (19); **Felippone F.** 2057 (13), 2086 (19), 2088 (19), 2708 (19), 2985 (19), 2989 (13), 3155 (19), 3236 (13), 3321 (19), 3394 (13), 3559 (3), 5472 (19), 5743 (19), 6003 (13), 6935 (19); **Fernández** 509 (13), Fernández et al. 85 (15); **Ferrucci M.** 186 (19), 841 (3); Ferrucci et al. 1176 (19); 1278 (19), 6426 (13); **Fidao** 1826 (2); **Fiebrig K.** 502 (20), 5645 (20), 5884 (18), 6305 (21); **Fischer W.** 236 (3); **Flessderf A.** 15916 (13); **Flossdorf A.** 12 (3), 47 (11), 175 (21), 3823 (11); **Fortuna** 76 (13); **Fortunato R.** 101 (11), 185 (13), 2582 (11); Fortunato et al. 231 (13), 1378 (11), 1574 (15), 1668 (15), 2299 (3); 2408(1/2) (13), 6087 (3), 6821 (11); **Fraga A.** 18 (15); **Franceschi F.** 131 (3), 498 (11), 638 (15), 646 (2); **Frangi J.** 143 (13); **Fryxell** 1769 (14); **Funck** 54 (15). **Galander C.** s.n. (14); **Galli** 141 (19), 290 (13); **Gallinal J.** et al. 1611 (19); **Gamerro** 38 (18); **Garaventa A.** 1661 (15); **García** s.n. (3); **Garibaldi** 174 (15); **Garzón T.** 2466 (13); **Gay C.** 794 (15); **Gemiguani** 23560 (13); **Gentry A.** et al. 44367 (10), 59266 (2); **Gianetti** 3426 (21); **Giardelli M.** 93 (14), 99 (19), 143 (11), 244 (19), 1053 (13), 1161 (3), 19537 (11); **Gimate** 625 (15); **Giménez A.** 118 (13); **Girardi A.** et al. ICN 21864 (15); **Glaziou** 17716 (16), 18391 (6); **Gonzalez** & Mora 512 (3), 941 (15); **Gorostegui** 125 (3); **Goulding** 713 (3); **Grazziotin** et al. 550 (15); **Grondona E.** & Spegazzini 1292 (21); **Guaglianone R.** et al 227 (19), 232 (20), 233 (13), 369 (3), 577 (15), 1435 (11), 1593 (11), 1807 (11), 2078 (15), 2962 (21), 3273 (2), 3274 (13), 3275 (3), 3276 (14), 3277 (13), 3279 (2), 3280 (15); **Gurni-Wagner** s.n. BAF 8848 (19). **Haene E.** 449 (15); **Hagelund K.** 132-40 (21), 132-43 (20), 132-44 (3), 2121 (15), 4741 (18), 5919 (19), 6525 (19), 7121 (13), 8875 (16), 8904 (8), 9623 (19), 10167 (20), 10168 (20), 10664 (22), 12174 (16), 14314 (22), 14654 (21); **Hahn** et al. 924 (15); Hahn 1947 (3); **Handro O.** 607 (9); **Harley R.** et al. 28030 (21), 28106 (2); **Harris W.** 11969 (21); **Hassler E.** 45 (15); **Hatschbach G.** 599 (9), 2672 (6), 2873 (18), 2896 (19), 8866 (19), 11539 (15), 11756 (6), 14781 (9), 14883 (23), 14898 (23), 14982 (8), 15669 (9), 17101 (8), 17252 (12), 18234 (18), 18439 (9), 18711 (9), 19753 (18), 20073 (8), 20082 (6), 20157 (22), 20177 (1), 20239 (1), 20479 (21), 20783 (9), 22284 (4), 22316 (5), 22483 (16), 22487 (9), 22571 (9), 22719 (6), 22732 (18), 22840 (9), 22859 (6), 23914 (9), 24137 (5), 24883 (19), 25327 (16), 25403 (8), 25406 (5), 25608 (9), 25632 (21), 25843 (9), 26225 (6), 26549 (6), 28218 (20), 28315 (6), 32787 (21), 33830 (21), 33830 (21), 40471 (6), 41138 (2), 52321 (18). **Hauman L.** BA nro. 24/2146 (13), BA 24/530 (20), 23540 (21), 23671 (20), 23714 (13); **Henz E.** s.n. (18), 35346 (19); **Hermann P.** 4691 (15); **Herrera J.** 17 (14); **Herter W. G.** 268 (3), 269 (19); **Hicken C. M.** 19 (13), 111 (14), 112 (15), 113 (15), 114 (15), 116 (11), 462 (13), 475 (13), 517 (13), 628 (3), s.n. (13), s.n. (13), SI nro. 3335 (3), SI nro. 3400 (15), SI nro. 3424 (21), SI nro. 3428 (21), SI nro. 3437 (13), SI nro. 3803 (15), SI nro. 3804 (19), SI nro. 3805 (15), SI nro. 3807 (19), SI nro. 3798 (3), SI nro. 3808 (11), SI nro. 3826 (13), SI nro. 3829 (13), SI nro. 8760 (3), SI nro. 10519 (11); **Hilgert** 518 (2); **Hoehne F.** 15152 (3), SPF 11225 (19); **Hollermayer A.** 1362 (14); **Holmberg E.** 357 (19); **Hosseus C.** 516 (11); **Huajardo** 4840 (13); **Humbert** et al. 27159 (15); **Hunziker A. T.** 1743 (11), 6020 (11), 8619 (13), 9417 (13), 9477 (19), 15934 (21), 18986 (3), 23615 (11); Hunziker A. T. & Di Fulvio 17132 (11), 21387 (13), 21425 (13); Hunziker A. & Cocucci 17614 (11); A. Hunziker et al. 21589 (15). **Hunziker J.** 39 (3), 500 (2), 7240 (15), 8211 (10), 9952 (21); Hunziker J. & Gamerro 11529 (13); Hunziker J. et al. 10755 (3), 10759 (15), 10847 (15), 11011 (11), 11025 (15), 11053 (15), 11054 (3), 12226 (15); 12790 (15); **Hunziker J.** & M. D. Hunziker 13223 (21); **Hurrel J.** 63 (15), 1264 (19); Hurrel & Belgrano 2788 (13); Hurrel et al. 1013 (15), 1176 (15), 1262 (13), 1586 (13), 1723 (14), 1727 (3), 2116 (19), 2130 (15), 2131 (19), 2134 (13), 2138 (3), 2152 (13), 2200 (14), 2206 (19), 2222 (13), 2224 (3), 2286 (3), 3675 (13), 3765 (3), 3766 (11), 5016 (3), 5206 (15). **Illin N.** s.n. (21); **Imaguire N.** 39 (9), 85 (9); **Insfrán** 939 (21); **Irigoyen J.** 1 (21), 38 (3), 76 (5); **Isez.** I. W. 130 (15). **Job M.** 796 (3), BA 65067 (13); **Johnson D.** 827 (15); **Jolly** 341ABJ (14), 342ABJ (3); **Jøergensen P.** s.n. (15), 508 (14), 1021 (11), 1294 (15), 1295 (3), 2464 (21), 2468 (3), 2638 (11), 3408 (13), 3767 (15); 3770 (21), 3768 (3), BAB 30578 (20), BAB 34548 (20); **Jurado** BA 56967 (3), BA 25/364 (19). **Keel** & Spinzi 1468 (5); **Keller H.** & Acosta 2563 (3); **Kiesling R.** 5530 (15); Kiesling & Ferrari 8527 (15); Kiesling et al. 1612 (11), 3600 (11), 3797 (10), 3955 (11), 4948 (15), 5087 (11), 5140 (15), 5403 (15), 5492 (11), 5889 (11), 9165 (13); **King D. 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M.** 2227 (11), 2850 (15) Molina & Sánchez 2433 (3), 2473 (3); Molina et al. 1603 (15), 1764 (15); **Montero G.** s.n. (15); **Montes J. E.** 91 (15), 802 (21), 3334 (3), 3921 (15), 4308 (21), 10351 (15), 15066 (15), 15076 (22), 15112 (22), 15107 (15), 15124 (19), 15171 (15), 15193 (15), 15216 (19), 15415 (21), 15448 (21), 15452 (19); **Monticelli** C-17 (13); **Morais H. C.** s.n. (3); **Morello** 4042 (3), SI nro. 16914 (3); **Morrone O.** 887 (19); Morrone et al. 590 (21), 601 (15), 1344 (18), 1844 (2), 4378 (11), 4391 (15), 4393 (15), 4402 (15), 4614 (15), 5300 (5); Morrone & Belgrano 4815 (10); Morrone & Pensiero 302 (21), 411 (15); **Mroginski L.** 188 (19), Mroginski 248 (19); Mroginski et al. 513 (21). **Múlgura M. E.** 1117 (15), 3487 (13), 3788 (3); Múlgura & Deginani 104 (14); Múlgura et al. 490 (19), 1555 (21), 2120 (19), 2199 (3), 2252 (22), 2400 (13), 2774 (11), 2780 (15), 2922 (13), 2931 (19), 3028 (3), 3039 (19), 3638 (15), 3753 (15), 3773 (15), 3776 (21), 3796 (13), 4050 (21). **Münch** 991 (19). **Naranjo C.** 756/26 (19), 759 /22 (21), 764 /30 (13); Naranjo et al. 873 (15); **Nee M.** 30410 (11), 33552 (15), 33740 (15); Nee M. & D. Atha 50079 (15); Nee M. & K. Taylor 26318 (15); Nee M. et al. 43959 (14); **Nicora E.** 159 (3), 574 (15), 976 (11), 1547 (19), 1574 (13), 1988 (21), 2006 (19), 2292 (13), 3277 (13), 3280 (19), 3283 (14), 3473 (3), 4761 (19), 4866 (13), 5183 (21), 5221 (5), 5287 (3), 6084 (15), 6344 (3), 6391(5), 6515 (2), 9928 (15), SI 18598 (13); **Niederlein G.** 541 (20); **Novara L.** 1905 (10), 2617 (11), 6858 (15), 7313 (11); Novara & Bruno 9412 (13); **Nuncia Tur** et al. 1785 (13). **Olea D.** 134 (3); **O'Leary N.** 122 (19), 123 (14), 501 (15), 502 (15), 503 (19), 504 (19), 505 (19), 506 (19), 507 (3), 510 (19), 511 (19), 512 (19), 514 (13), 525 (13), 526 (15), 527 (15), 528 (15), 529 (14), 530 (14), 531 (19), 532 (15); O'Leary et al. 600 (3), 609 (3), 610 (3), 613 (11), 615 (13); **Olmstead R.** et al. 2004-104 (3), 2004-105 (15), 2004-106 (13), 2004-111 (21), 2004-112 (19), 2004-144 (2); **Osten C.** 5391 (2), 16049 (21). **Pabst G. F.** s.n. (15), 4605 (21), 5425 (15), 5490 (13), 5868 (18), 6140 (1), 6144 (8); **Partridge W.** BA 43074 (14), BA 59560 (13), BA 59710 (19), BA 60332 (13), BA 61175 (14), BA 61895 (2); **Pastore A. L.** 49 (13), 1140 (13), 1147 (19), 1229 (19), 2034 (14), 2054 (3); **Pavetti C. V.** & Rojas (3); **Pedelaborde J. L.** 12127 (19); **Pedersen T. M.** 774 (15), 3831 (19), 4018 (11), 4341 (3), 4660 (13), 5074 (19), 5205 (21), 5435 (13), 5540 (5) 6144 (2), 6238 (2), 6309 (19), 6417 (15), 6493 (5), 7149 (19), 7346 (15), 7680 (13), 8127 (15), 8625 (15), 9257 (2), 9816 (19), 9867 (11), 10952 (9), 11485 (8), 12005 (13), 12973 (21), 13500 (14), 14105 (14), 16189 (19), CTES 350152 (20), CTES 350510 (21), CTES 354599 (22); **Pellani** s.n. (3); **Pelosi** 18 (13); **Pensiero J. F.** 709 (3), 1137 (3), 1745 (13), 6460 (15), 6462 (3); Pensiero & Exner 5943 (13); Pensiero & Fauvre 2312 (3); Pensiero & Marino 4556 (15); Pensiero & Tivano 3306 (3); Pensiero & Vegetti 2563 (15); **Pereyra** 13 (15); **Pereira E.** 4897 (15), 5187 (21), 5238 (9), 5409 (9), 5481 (9), 7941 (9), 7954 (8), 8125 (8); **Pérez L.** 243 (15), 486 (11); Pérez & Milan Vavrek 1 (15); **Pérez** B. 116 (14); **Pérez Moreau** 3860 (13), 8316 (3); BA 31/2242 (21), BA 44015 (3); Pérez Moreau & Petetin 3832 (15), 3973 (11); Pérez Moreau & Perrone BA 30140 (13); **Perrone V.** 7024 (13), 54567 (11), 54569 (13); **Petetin C. A.** 1672 (11); Petetin et al. 1796 (15); **Piccinini B.** & Leguizamón 2013 (11); 2603 (11); Piccinini & Petetin 3131 (11), 3133 (15), 3341 (11); **Pierotti** 5144 (15); **Pire S. M.** 2231 (3); Pire & Mroginski 182 (21); **Pogarzo** & Gandolfo 90 A 136 (3); **Pozzi** BA nro. 27/1803 (13); **Prina A. O.** et al. 2442 (11); **Purpus C. A.** 6413 (21); **Puyssegur** s.n. 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M.** 2826 (14), 10241 (15); **Ricter** 5606 (18); **Riedel L.** 110 (11); **Rimbeth** 20 (19); **Ritcher** s.n. (21); **Rodrigo** 950 (15), 2363 (15), 2432 (11); **Rodríguez F.** 5 (2), 93 (15), 135 (15), 161 (11), 223 (15), 471 (3), 545 (21), 570 (6), 596 (20); Rodriguez R. et al. 869 (14); **Rojas T.** 241-A (2), 1877 (14), 1880 (2), 1887 (15), 1890 (11), 1892 (3), 2514 (11), 2519 (14), 2520 (11), 2522 (15), 3365 (21), 3407 (21), 4819[a] (12), 4819 (2), 5109 (21), 5788 (20), 6128 (20), 8666[a] (3), 8666 (3), 8893 (11), 9278 (12), 9463 (21), 13559 (3); **Romanczuk M. C.** et al. 355 (13), 395 (19), 433 (21); **Rosa E. B.** 1340 (13); **Rosa-Mato** 636 (2); **Rosengurtt B.** B730 (19), B783 (2), B864 (2), B865 (15), B1016 (15), B1915 (13), B1920 (21), 535 (13), 765 (13), 1002 (13), 1269 (19), 2513 (13); **Rossow R. A.** 2543 (14), 3074 (15); Rossow & Gómez Cadret 1805 (13); **Rotman A.** 802 (11), 1023 (11); Rotman & Ahumada 1101 (11), 1105

(19), 1107 (15); Rotman et al. 94 (14), 262 (15); **Rotours** BAB 66590 (14); **Rua G. H.** 20880 (19); **Rúgolo Z.** et al. 812 (13); **Ruiz L.** 594 (15); **Ruiz Huidobro A. M.** 1473 (11), 2087 (19); **Ruiz Leal R. A.** 1046 (13), 1498 (13), 2026 (11), 3054 (11), 3055 (13), 4525 (15), 8500 (13), 9328 (11), 9337 (11). **Sabbatke** Neto s.n. (19); **Sagástegui A.** & Aguado 2603 (7), 7192 (15); Sagástegui et al. 6163 (7), 10377 (10); **Saito Y.** 436 (16); **Salgado C. R.** 125 (3); **Sammler** s.n. (15); **Sanchez Vega I.** 272 (7), 853 (7); **Sánchez** et al. 169 (15); **Saravia Toledo C.** 2879 (11), 14685 (11); Saravia Toledo et al. 13130 (15); **Sayago M.** 989 (14), 2454 (3); **Schinini A.** 3453 (21), 3455 (21), 5361 (14), 7281 (19), 7793 (14), 8309 (13), 13995 (21), 16105 (3), 16786 (2), 35259 (5); Schinini & Ahumada 13927 (15); Schinini & Caballero Marmori 30051 (21); Schinini & Carnevali 10297 (20), 10471 (15), 10472 (13), 10533 (20), 10689 (13); Schinini & Cristóbal 9707 (19), 13650 (5); Schinini & Mroginski 4618 (5); Schinini & Quarin 14418 (3), 14458 (5); Schinini et al. 6248 (13), 7281 (19), 7398 (2), 7403 (19), 10259 (15), 11779 (21), 16736 (3), 16803 (2), 19176 (13), 19257 (3), 19290 (21), 31322 (11), 31325 (3), 35050 (2), 35453 (20), 35698 (19), 35726 (13); **Schmeda** 290 (21), 291 (19); **Schnack B.** 2670 (3), IF2332bis (13); **Schrottky C.** 14 (21); **Schulz F.** 6 (3), 99 (3), 149 (13), 150 (11), 151 (3), 152 (15), 1480 (21), 3557 (21), 4155 (15), 5598 (20), 6387 (11), 6986 (20), 7682 (5), 7808 (3), 8374 (13), 8755 (15), 8757 (3), 8794 (13), 9055 (21), 10031 (3), 10839 (21), 11122 (15), 13745 (14), 15036 (13), 17665 (3), 17688 (21), 17776 (11); **Schnem A.** 3497 (18), 3519 (15), 3958 (18), 4981 (18), 5130 (8), 5131 (6), 5155 (8), 5449 (18); **Seijó E. J.** 1334 (13), 1366 (13); **Smith L. B.** & Klein 7504 (16), 7787 (16), 8065 (1), 8101 (8), 8105 (18), 8428 (9), 10508 (17), 11041 (6), 11107 (8), 11115 (20), 11401 (6), 11445 (9), 11968 (21), 12118 (9), 13021 (9), 13333 (6), 13390 (6), 13394 (9), 13469 (16), 13554 (9), 13733 (9), 13740 (6), 13870 (6), 13885 (9), 13968 (3), 14102 (15), 14936 (9); Smith & Reitz 9059 (1), 9122 (6), 9146 (16), 9162 (9), 9226 (9), 9730 (19), 9839 (9), 10024 (8), 10175 (16), 10185 (8), 10303 (16), 12482 (6), 12831 (6), 13390 (6), 14204 (8), 14370 (6), 14330 (13); Smith et al. 8696 (23), 8916 (23), 9315 (9); 9371 (9), 9463 (16), 9544 (6), 9545 (3), 9568 (6), 9624 (15), 10341 (23), 14526 (8), 14562 (9); **Soares** 242 (22); **Sobral M.** 1246 (6), 1251 (8), Sobral & Grabauska 3937 (2), Sobral et al. 4837 (8); **Solis Neffa V.** et al. 87 (13), 288 (21); **Solomon J. C.** 7151 (11), 8601 (15); 11467 (12/13), 12759A (10), 12759 (11), 13254 (10), 15028 (11), 16447 (10), 17441 (10), 17545 (10); Solomon & King 15964 (3); **Soria** 73 (15), 419 (15), 5491 (15), 5530 (3), 8101 (15); **Soriano A.** 563 (11); **Soukup J.** 259 (11); **Soza V.** 1815 (15), 1816 (11), 1821 (15), 1826 (11); **Spegazzini C. L.** s.n. (11), 17 (15), 187 (2), BAB 6442 (13), BAB 14654 (15), BAB 17626 (22); **Steibel P.** 903 (10), 2193 (13), 2459 (13), 3999 (3); Steibel et al. 3242 (13); **Steinbach J.** 84 (11), 2157 (15), 2368 (15), 6009 (10), 6052 (21), 9681 (14), BA 27/625 (15); **Stella Soraní** s.n. (19), 48 (3), 116 (13); **Subils R.** 706 (3), 2172 (15), 2286 (15), 2381 (13), 2531 (15), 3748 (13), 23027 (15); Subils R. & Bernardello 2370 (14). **Tolaba J. A.** 3272 (15); **Tremoleras** s.n. SI 3849 (21); **Tressens S. G.** 6593 (13); Tressens et al. 532 (14), 1875 (20), 2549 (19), 2691 (20), 3652 (3), 3712 (2), 6056 (21); **Troiani H. O.** & Steibel 3851 (13), 3919 (14), 4182 (13); Troiani et al. 3718 (13); **Troncoso N. S.** 185 (19), 319 (11), 360 (2), 986, 987 (15), 1246 (13), 2830 (2), 6025 (13), 19544 (19), 19545 (14), Troncoso et al. 1092 (13), 2043 (13), 2725 (2), 3584 (13), 3780 (15). **Ule E.** 1068 (1); **Ulibarri E.** 1521 (14). **Vanni R. O.** et al. 748 (19), 2097 (11), 3836 (3), 3838 (19); **Vegetti** 166 (14); Vegetti & Pensiero 728 (13); **Velarde O.** 670 (7), 1664 (11); **Venturi S.** 5 (21), 14 (15), 50 (15), 53 (11), 54 (2), 68 (19), 84 (3), 123 (3), 273 (21), 520 (11), 525 (11), 825 (11), 1171 (3), 2767 (14), 2814 (3), 2936 (3), 2937 (3), 3702 (14), 5370 (11), 7076 (15), 7229 (11), 7984 (3); **Vera N.** s.n. (11), 137 (11); **Vervoorst F. B.** et al. 4253 (15); **Vignati** 7029 (13), 7031 (13), SI 25348 (3); **Viullieumier** 333 (11). **Widgren** s.n. (6); **Williamson J.** 2900 (3); **Wolf N. M.** et al. 55 (15); **Woodbury** & García Llano s.n. (15); **Woolston A. L.** 107 (15), 174 (14), 785 (2), 916 (20). **Yoshiko** Kuniyoshi 3327 (6); **Yosisaki** s.n. (19). **Zanardini I.** s.n. (18); **Zardini E.** 1014 (19), 5040 (15), 8706 (21), 11219 (3), 15277 (15), 17030 (15), 18649 (14); Zardini & Aguayo 10547 (19); Zardini & Gamarra 59223 (20), 59236 (19), 59238 (3), 59262 (20), 59784 (3), 59817 (3); Zardini & Guerrero 58643 (21); Zardini & Perez 2832 (15); Zardini & Salinas 24500 (15); Zardini & Velásquez 10164 (3), 10177 (15), 11568 (15), 16051 (3), 22708 (15), 24656 (15), 25220 (19), 26656 (15); Zardini et al. 1086 (2), 9192 (3); **Zuberbühler** 57265 (15); **Zuloaga F. O.** 1743 (11), 8266 (11), 8280 (21), Zuloaga et al. 749 (15), 1020 (15), 1395 (15), 2069 (19), 2248 (19), 2319 (21), 2320 (15), 2325 (21), 3209 (21), 3222 (13) 3224 (13), 3296, (21), 3332 (21), 3618 (11), 3968 (15), 5030 (15), 5377 (21), 5452 (15); Zuloaga, F. O. & Belgrano, M. J. 8093 (13), 8105 (19), 8111 (21), 8112 (19), 8121 (21), 8117 (13), 8150 (15), 8203 (3), 8335 (21), 8350 (21), 8414 (21); Zuloaga & Deginani 3800 (15); Zuloaga & Morrone 7059 (15).

Apéndice 3. Lista de las especies sinonimizadas en este trabajo con indicación de su actual ubicación taxonómica. La numeración de los taxones en los Apéndices 1 y 2 únicamente se aplica en los mismos y difiere de la usada en el resto del texto.

Verbena alata f. *alba* Moldenke nuevo sinónimo para *V. lindbergii* Moldenke

Verbena australis Moldenke nuevo sinónimo para *V. filicaulis* Schauer

Verbena bangiana Moldenke nuevo sinónimo para *V. hispida* var. *oborata* (Moldenke) N. O'Leary

Verbena bonariensis f. *albiflora* Moldenke nuevo sinónimo para *V. bonariensis* L. var. *bonariensis*

Verbena bonariensis L. f. *robustior* Chodat nuevo sinónimo para *V. ovata* Cham.

Verbena bonariensis var. *hispida* Moldenke nuevo sinónimo para *V. intercedens* Briq.

Verbena brasiliensis var. *subglabrata* Moldenke nuevo sinónimo para *V. litoralis* var. *brevibracteata* (Kuntze) N. O'Leary

Verbena cordobensis Briq. nuevo sinónimo para *V. litoralis* Kunth var. *litoralis*

Verbena ephedroides var. *entreriensis* Tronc. nuevo sinónimo para *V. ephedroides* Cham.

Verbena filicaulis var. *australis* (Moldenke) Moldenke nuevo sinónimo para *V. filicaulis* Schauer

Verbena hirta var. *dusenii* Moldenke nuevo sinónimo para *V. hirta* Spreng. var. *hirta*

Verbena inamoena Briq. nuevo sinónimo para *V. bonariensis* var. *conglomerata* Briq.

Verbena integrifolia Sessé & Moç. f. *albiflora* Moldenke nuevo sinónimo para *V. litoralis* Kunth var. *litoralis*

Verbena intermedia f. *albiflora* Moldenke nuevo sinónimo para *V. intermedia* Gillies & Hook. ex Hook.

Verbena intermedia f. *glabrescens* Hauman nuevo sinónimo para *V. litoralis* var. *brevibracteata* (Kuntze) N. O'Leary

Verbena intermedia var. *lanuginosa* Moldenke nuevo sinónimo para *V. hispida* Ruiz & Pav. var. *hispida*

Verbena isabellei Briq. nuevo sinónimo para *V. montevidensis* Spreng.

Verbena lindmanii Briq. nuevo sinónimo para *V. sagittalis* Cham.

Verbena litoralis f. *angustifolia* Chodat nuevo sinónimo para *V. litoralis* Kunth var. *litoralis*

Verbena litoralis f. *congesta* (Moldenke) Moldenke nuevo sinónimo para *V. litoralis* var. *brevibracteata* (Kuntze) N. O'Leary

Verbena litoralis var. *congesta* Moldenke nuevo sinónimo para *V. litoralis* var. *brevibracteata* (Kuntze) N. O'Leary

Verbena litoralis var. *melanopotamica* Hauman nuevo sinónimo para *V. intermedia* Gillies & Hook. ex Hook.

Verbena litoralis var. *portoricensis* Moldenke nuevo sinónimo para *V. litoralis* Kunth var. *litoralis*

Verbena lobata var. *hirsuta* Moldenke nuevo sinónimo para *V. lobata* Vell. var. *lobata*

Verbena malmei Moldenke nuevo sinónimo para *V. lindbergii* Moldenke

Verbena minutiflora Briq. ex Moldenke nuevo sinónimo para *V. montevidensis* Spreng.

Verbena minutiflora var. *peruviana* Moldenke nuevo sinónimo para *V. montevidensis* Spreng.

Verbena paranensis Moldenke nuevo sinónimo para *V. hirta* Spreng. var. *hirta*

Verbena parvula f. *albiflora* Moldenke nuevo sinónimo para *V. hispida* Ruiz & Pav. var. *hispida*

Verbena parvula Hayek nuevo sinónimo para *V. glabrata* Kunth

Verbena parvula Hayek var. *gigas* Moldenke nuevo sinónimo para *V. glabrata* Kunth

Verbena paucifolia Turcz. nuevo sinónimo para *V. litoralis* var. *brevibracteata* (Kuntze) N. O'Leary

Verbena regnelliana Moldenke nuevo sinónimo para *V. filicaulis* Schauer

Verbena reitzii Moldenke nuevo sinónimo para *V. lindbergii* Moldenke

Verbena reitzii var. *castrensis* Moldenke nuevo sinónimo para *V. hirta* var. *gracilis* Dusén

Verbena rigida f. *obovata* Hayek nuevo sinónimo para *V. rigida* Spreng.

Verbena rigida f. *paraguayensis* Moldenke nuevo sinónimo para *V. rigida* Spreng.

Verbena rigida var. *glandulifera* (sphalm. *glandulosa*) Moldenke nuevo sinónimo para *V. rigida* Spreng.

Verbena rigida var. *obovata* (Hayek) Moldenke nuevo sinónimo para *V. rigida* Spreng.

Verbena rigida var. *reineckii* (Briq.) Moldenke nuevo sinónimo para *V. rigida* Spreng.

Verbena sphaerocarpa L. M. Perry nuevo sinónimo para *V. litoralis* Kunth var. *litoralis*

Verbena strigosa Cham. nuevo sinónimo para *V. hirta* var. *gracilis* Dusén

Verbena venosa var. *reineckii* Briq. nuevo sinónimo para *V. rigida* Spreng.

A REVISION OF THE GENUS *PITHECOCTENIUM* (BIGNONIACEAE)[1,2]

Amy Pool[3]

ABSTRACT

Pithecoctenium Mart. ex Meisn. is a genus of four species in the tribe Bignonieae. All the species are lianas, and can be recognized by their acutely hexangular branchlets, bifoliolate leaves with a trifid or, more frequently, additionally trifurcately-branched terminal tendril, arching corollas that are coriaceous, white, and pubescent, and echinate fruits (except in *P. falcatum* (Vell.) A. Pool) with seeds surrounded on three sides by a clearly demarked, hyaline-membranous wing. Relationships with similar genera are discussed, and a key to the species of *Pithecoctenium*, their descriptions, and distribution maps are provided. The relationships of the species are also discussed. Lectotypes are designated for *Bignonia squalus* Vell., *P. echinatum* var. *tomentosum* Hassl., *P. vitalba* f. *glabratum* Hassl., and *P. vitalba* f. *intermedium* Hassl., a lectotype and epitype are designated for *B. falcata* Vell., and a new combination, *P. falcatum* (Vell.) A. Pool, is proposed. The following are presented as new synonyms of *P. crucigerum* (L.) A. H. Gentry: *B. botryoides* Cham., *B. vitalba* Cham., *B. vitalba* var. *extratropica* Cham., *P. catharinae* DC., *P. echinatum* var. *tomentosum* Hassl., *P. echinatum* var. *tomentosum* f. *grandiflorum* Hassl., *P. hexagonum* var. *brasilianum* DC., *P. vitalba* f. *glabratum* Hassl., *P. vitalba* f. *intermedium* Hassl., and *P. echinatum* var. *intermedium* Hassl. *Pithecoctenium cynanchoides* DC., *Anemopaegma clematideum* Griseb., and *P. cynanchoides* var. *pellucidum* Hassl. are presented as new synonyms of *P. carolinae* (Lindl.) G. Nicholson. *Pithecoctenium cordifolium* DC. (including the nomina nova for *P. cordifolium* DC.: *Neves-armondia cordifolia* K. Schum. and *P. hatschbachii* A. H. Gentry) is presented as a new synonym of *P. falcatum* (Vell.) A. Pool.

Key words: Bignoniaceae, Pithecocteniinae, *Pithecoctenium*.

Pithecoctenium Mart. ex Meisn. is a small Neotropical genus of four species in the tribe Bignonieae that has a number of vegetative, floral, and fruiting characters that make it fairly easy to recognize. As most members of the tribe Bignonieae, *Pithecoctenium* is a genus of lianas that has compound leaves with the terminal leaflet modified into a tendril, bilocular ovaries, and fruits with dehiscence parallel to the septum (Gentry, 1980). *Pithecoctenium* has branchlets without interpetiolar glandular fields, which are distinctly and acutely 6-angled with the ribs on the angles usually detaching with age, and leaves that are bifoliolate with a terminal tendril that is trifid or, more frequently, additionally branched. The calyx is truncate with the ribs extending apically to form five small, sometimes inconspicuous, denticles; the calyx and corolla are both very coriaceous, the corolla especially so at its glabrous base, above the base it is densely pubescent and the tube is generally bent at a right angle at a point 1/3 to 1/2 its length from the base. The ovary sits on a large nectary disc and is covered with dense thick trichomes, except in *P. falcatum* (Vell.) A. Pool, which is lepidote. The capsule is thick, somewhat compressed, oblong-elliptic, woody, and usually densely echinate or muricate (smooth in *P. falcatum*). The seeds have a large wing that surrounds the seed body on three sides, and to varying degrees on the side of attachment, and is shiny, hyaline-membranous, and primarily white or colorless.

Melchior (1927) treated *Pithecoctenium* in his subtribe Pithecocteniinae (in tribe Bignonieae, Bignoniaceae), which also included *Distictella* Kuntze, *Neves-armondia* K. Schum. [referred to *Pithecoctenium*, *P. falcatum* (Vell.) A. Pool], *Urbanolophium* Melch. [= *Haplolophium* Cham., fide Gentry, 1992], *Haplolophium* Cham., *Glaziovia* Benth. & Hook. f., and *Amphilophium* Kunth. The subtribe was described as having bifoliolate or trifoliolate leaves, tendrils usually trifid (rarely bifid), or additionally forked, and often with disk-like tips, the calyx thickly coriaceous and densely tomentose, with or without defined lobes,

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the corolla coriaceous, and except in *Amphilophium*, densely pubescent, often curved at a right angle and sometimes more or less bilabiate, and the capsule oblong-elliptic, flattened, the surface smooth or rough to echinate, and the seed with a membranous wing.

Amphilophium and most species of *Haplolophium* have branchlets that are acutely hexangular as in *Pithecoctenium*; however, they can be separated vegetatively from *Pithecoctenium* by their tendrils that are never more than 3-branched and by the presence of at least some branched trichomes (not found in *Pithecoctenium*). Both have calyces with expanded, frilly margins, quite different from that found in *Pithecoctenium*, and *Amphilophium* has a strongly bilabiate pseudo-cleistogamous corolla versus the weakly zygomorphic, open corollas of *Pithecoctenium*. The monotypic genus *Glaziovia* has a terete stem and flowers similar to those of *Amphilophium*. *Distictella* has flowers very similar to those of *Pithecoctenium*, very coriaceous, white, densely pubescent and strongly curved; however, its branchlets are terete and unribbed and the tendrils not more than 3-branched. Fruits and seeds have not been seen for all of the species in all the genera of Melchior's subtribe Pithecocteniinae; however, all examined materials have fruits that are woody, relatively broad, oblong-elliptic or elliptic, and biconvex to somewhat compressed or, in *Distictella*, often strongly curved with one convex and one concave surface. The valve surface is smooth to slightly roughened in *Distictella* and *Glaziovia* but can be smooth to tuberculate in *Amphilophium* and smooth to echinate in *Haplolophium*. The seeds of the non-*Pithecoctenium* species are more strongly transversely oblong, with the thickened zone surrounding the body extending nearly to the wing tips and thus appearing more bialate, that is, as a seed with two lateral wings. The wings are also darker and less clearly demarcated from the seed body. Melchior (1927) provided a key to the genera in the subtribe Pithecocteniinae; however, many new species have been described (Sandwith, 1954a, 1957; Gentry, 1978, 1992; etc.) and generic concepts have changed (Gentry, 1992). Nonetheless, within the Pithecocteniinae, *Pithecoctenium* seems most closely related to *Distictella* as suggested by Melchior's key.

Distictis Mart. ex Meisn., *Ceratophytum* Pittier, and *Clytostoma* Miers ex Bureau, genera not included in Melchior's subtribe, Pithecocteniinae, also share characteristics with *Pithecoctenium*. *Distictis* is the most similar to *Pithecoctenium*; flowering and vegetative specimens of *Distictis* are sometimes misidentified as *Pithecoctenium*, and many species of *Distictis*, for which fruits were not known, were originally described as *Pithecoctenium* (for example: *P. buccinatorium* DC. = *D. buccinatoria* (DC.) A. H. Gentry, *P. stipulare* Mart. ex DC. = *D. stipularis* (Mart. ex DC.) A. H. Gentry, *P. laxiflorum* DC. = *D. laxiflora* (DC.) Greenm., *P. scabriusculum* Mart. ex DC. = *D. scabriuscula* (Mart. ex DC.) A. H. Gentry). *Distictis* is similar to *Pithecoctenium* in having branchlets that are hexangular and ribbed, though usually less conspicuously so (but sometimes with the ribs detaching with age), and in having trifid (or sometimes more branched) tendrils. The flowers are white as in *Pithecoctenium* or purple, orange, or red, and the calyx is of similar shape and texture as that found in *Pithecoctenium*, but the corolla, while of the same general shape and pubescence, has a more or less straight tube and is of a somewhat more delicate texture. The fruit, like that of *Pithecoctenium*, is elliptic-oblong and woody, but is pointed at both ends, and the valves are never echinate or tuberculate. The seeds in some of the species approach those of *Pithecoctenium* in shape, and often have the wing well demarcated from the seed body, but the surface of the body is irregularly ridged. Based on these numerous vegetative, floral, and seed similarities, the relationship between these two genera should be further explored.

Ceratophytum has flowers that are very similar to those of *Pithecoctenium*, but is very different in many other aspects, such as terete branchlets with very conspicuous interpetiolar glandular fields, fruits oblong-linear and subtetragonal, and seeds bialate with the wings brown and not clearly demarcated from the seed body.

Fruiting specimens of *Clytostoma* are sometimes confused with *Pithecoctenium*. However, the echinate fruits of *Clytostoma* develop from ovaries that are covered with blunt, glandular projections while the echinate fruits of *Pithecoctenium* develop from ovaries that are densely sericeous with thick appressed trichomes. The fruiting projections of *Clytostoma* are curved while they are straight in *Pithecoctenium*, and the seeds of *Clytostoma* are thick, corky, and nearly wingless, or essentially winged on only one side. Gentry (1973a) described the resemblance of the fruit of the two genera as superficial, and suggested that the echinate projections evolved independently as a mechanism for protecting developing fruits and seeds from monkey predation.

Bureau and Schumann (1896) treated *Pithecoctenium falcatum* (under the illegitimate name, *P. cordifolium* DC.) in its own section, *Leiogyne*, within the genus *Pithecoctenium*, based on its glabrous (but lepidote) ovary and pellucid-punctate leaflets. The leaflets are actually pellucid-lepidote, a common characteristic in the genus *Pithecoctenium*. Schumann

(1897), after receiving a fruiting specimen of *P. falcatum*, published the new monotypic genus, *Neves-armondia*, and new combination *N. cordifolia* K. Schum., separating it from *Pithecoctenium* based on its non-pubescent ovary and smooth fruit. Melchior (1927) used these same characters (plus the pellucid-punctate leaflets) to separate *Neves-armondia* from *Pithecoctenium*. Gentry (1979) returned *N. cordifolia* to *Pithecoctenium* (as *P. hatschbachii* A. H. Gentry = *P. falcatum*) based on similar flower (excluding the glabrous ovary of *Neves-armondia*) and vegetative characters and identical pollen type (Gentry & Tomb, 1979). This placement is followed here and is further supported by the capsule of *P. falcatum*, which in its overall appearance, except for the surface, is similar to that of *P. crucigerum* (L.) A. H. Gentry, and by its seeds, which are also very similar. Furthermore, as both smooth and echinate or tuberculate fruits are found within the same genus in other genera in the Pithecocteniinae (*Amphilophium* and *Haplolophium*), this difference does not seem worthy of generic recognition.

History

Meisner established the genus *Pithecoctenium* in 1840, crediting Martius with the generic name. He made no new combinations but referred to de Candolle's unpublished *Prodromus* treatment (21 species), and cited published plates of *Bignonia echinata* Jacq. and *B. squalus* Vell., and text pages of *B. vitalba* Cham. and *B. botryoides* Cham. *Pithecoctenium echinatum* (Jacq.) Baill. (as K. Schum.) was later selected as the lectotype by Sandwith (1962).

Martius (1841) published the first combination in *Pithecoctenium*, *P. cordifolium* Mart., unfortunately an illegitimate name due to the citing of *Bignonia squalus* in synonymy. De Candolle (1845) recognized 21 species in his treatment of *Pithecoctenium* for the *Prodromus*, 17 as new species, three as new combinations, and one previously published by Splitgerber. Of these, 10 are still considered to be *Pithecoctenium*, with eight treated here as synonyms of *P. crucigerum*, six treated as *Distictis*, two as *Distictella*, and one each as *Arrabidaea* DC., *Clytostoma*, and *Paragonia* Bureau. Klotzsch (1848) published two new names (without diagnosis) and one new combination in *Pithecoctenium*. Miers (1863) recognized a relationship between *Haplolophium* and *Pithecoctenium* and suggested that *P. elongatum* (Vahl) Klotzsch (now treated within *Distictella*; Gentry, 1982a) belongs to neither of these genera, and published a new genus *Phaedranthus* Miers for *P. buccinatorium* (now treated within *Distictis*; Gentry, 1973a). Miers (1863: 183) also noted that "It will probably be found that some other Mexican species of *Pithecoctenium* enumerated by de Candolle, especially those with purple flowers and a 3-fid cirrhus, belong to this genus." Bureau, in his monograph of the family (1864), gave the first indication of a relationship between *Distictis* (as *Macrodiscus* Bureau) and *Pithecoctenium*.

Schumann (1894) published the new species *Pithecoctenium glaziovii* Bureau ex K. Schum. (now treated within *Haplolophium*; Gentry, 1992) and two combinations, *P. dolichoides* (Cham.) Bureau ex K. Schum. and *P. echinatum* (Jacq.) K. Schum. (although the latter had been made earlier by Baillon [1891], it is the Schumann publication that is most frequently cited, with the name incorrectly ascribed to Schumann), and in his key to genera placed *Pithecoctenium* in a trichotomy with *Haplolophium* and *Distictella* (as *Distictis* sensu Bureau), suggesting a relationship among the three. Bureau and Schumann (1896) placed many of de Candolle's names in the synonymy of *P. vitalba* (Cham.) DC. and *P. echinatum*, treated the three *Pithecoctenium* species of Klotzsch as *Distictis* sensu Bureau [= *Distictella*], moved other de Candolle species of *Pithecoctenium* to other genera, and recognized a new section in *Pithecoctenium*. Their treatment of the genus is very close to that followed here. They noted that *P. scabriusculum* Mart. ex DC. and *P. stipulare* Mart. ex DC. (both now treated as *Distictis*) were retained in *Pithecoctenium* with doubt, and that *P. echinatum*, *P. vitalba*, and *P. botryoides* (Cham.) DC. might all be one species. If *P. scabriusculum* and *P. stipulare* are removed from their treatment, *P. echinatum*, *P. vitalba*, and *P. botryoides* are all treated as one species (under the name *P. crucigerum*), and *P. cordifolium* DC. (a later homonym of *P. cordifolium* Mart.) is treated as *P. falcatum*, then there is little difference between the two treatments.

In 1897, Schumann established the monotypic genus *Neves-armondia*, with a single species, *N. cordifolia*, for *Pithecoctenium cordifolium* DC., based on its glabrous ovary and smooth fruit. Gentry (1979) moved this species back to *Pithecoctenium* under the new name *P. hatschbachii*. In this paper, it is treated as synonymous with *P. falcatum*.

Hassler (1910) recognized the variation in the now accepted *Pithecoctenium crucigerum* by providing a key to separate *P. echinatum* and *P. vitalba* and publishing a number of infraspecies in both. Melchior (1927) published the subtribe Pithecocteniinae and provided descriptions of, and a key to, the genera.

In 1975, Gentry (1975a) lectotypified *Bignonia crucigerum* L. and published *Pithecoctenium crucigerum* (L.) A. H. Gentry, the oldest name for what was

known as *P. echinatum* (including *P. vitalba*, etc.). In most modern Neotropical floras prior to 1975 (*Flora of Suriname*, Sandwith, 1938; *Flora of Trinidad and Tobago*, Sandwith, 1954b; *Flora of Peru*, Macbride, 1961; *Flora Argentina*, Fabris, 1965; *Flowering Plants of Jamaica*, Adams, 1972; *Flora of Panama*, Gentry, 1973b; *Flora Ilustrada Catarinense*, Sandwith & Hunt, 1974; *Flora of Guatemala*, Standley & Williams, 1974), this species is referred to as *P. echinatum*. Recent authors (Sandwith, 1937, 1938, 1954a; Fabris, 1965; Gentry, 1973a, 1973b, 1974, 1979, 1982a; Sandwith & Hunt, 1974; Barrie, 1991) have selected a number of lectotypes and placed numerous names in synonymy.

Anatomy

Santos (1995) studied 31 genera of Bignonieae and divided them into four groups based on the type of cambial variant present. Two species of *Pithecoctenium*, *P. crucigerum* and *P. dolichoides*, were included in her study. *Pithecoctenium* falls into her Group 1, which is characterized by stems with discontinuous phloem wedges intersected with xylem and parenchyma (Santos, 1995). The only other genus in this group is *Distictella*. *Pithecoctenium* differs from *Distictella* (and the other genera studied) by having secondary xylem in the bark (Santos, 1995). Two other genera in the Pithecocteniinae, *Amphilophium* and *Haplolophium*, as well as *Distictis* (among a number of other genera) fell into her Group 2, having stems with multiples of four phloem wedges in transverse section. The remaining genus in Pithecocteniinae, *Glaziovia*, was not included in her study.

Palynology

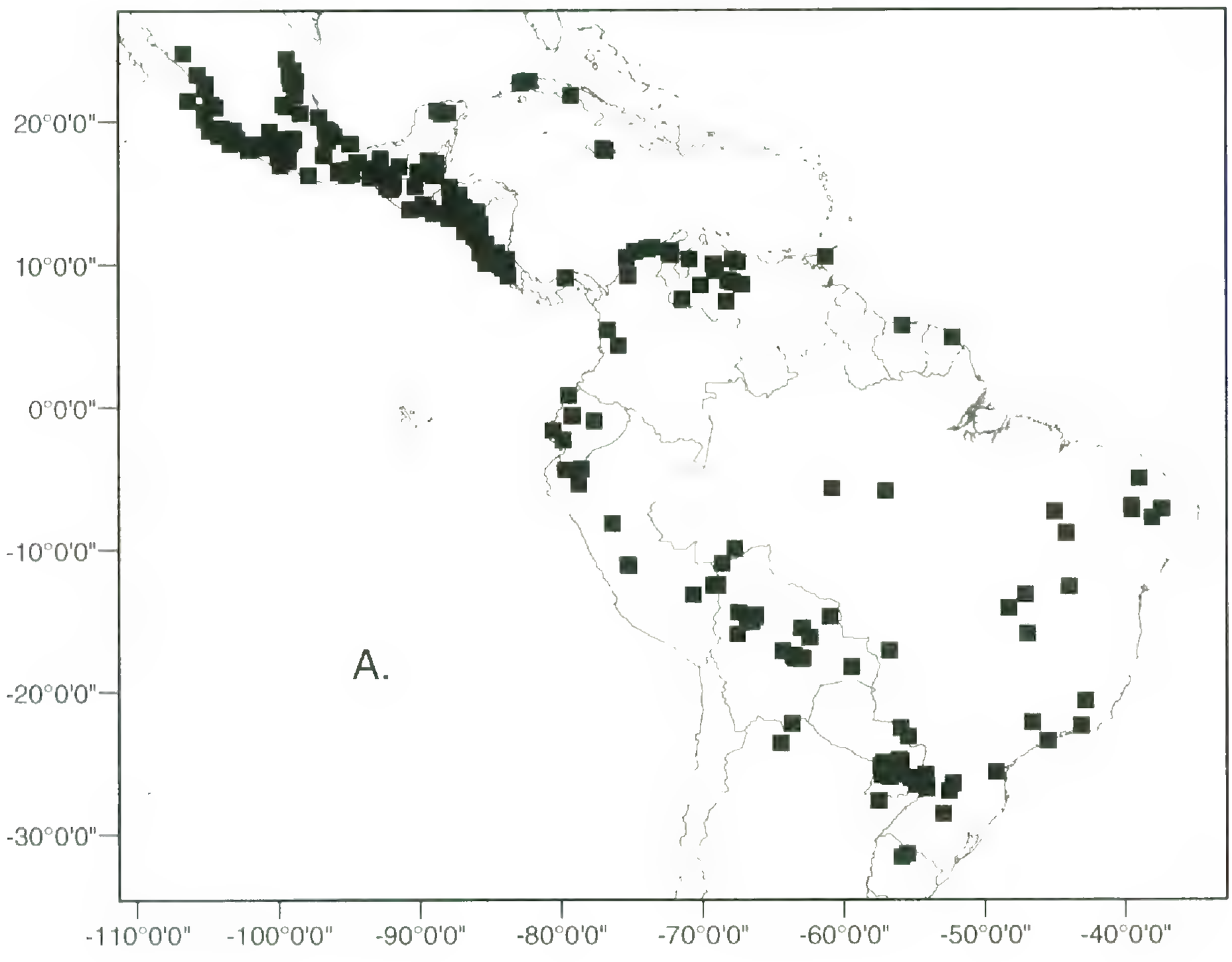
Pithecoctenium (represented by *P. crucigerum* and *P. falcatum*, as *P. hatschbachii*) has inaperturate, coarse-reticulate pollen (Gentry & Tomb, 1979: fig. 54). This pollen type was found in a number of other genera within the Bignoniaceae, which Gentry and Tomb (1979) suggested could be divided into two natural groups. The one including *Pithecoctenium* is characterized by having thick-textured white to purple or red flowers, trifid to multi-trifid tendrils, and multiseriate ovules and seeds. The other genera in the group are *Distictis* and *Distictella*. Members of the other group have medium-textured, yellow (rarely white or orange) flowers, simple tendrils, and mostly biseriate ovules and seeds; this group includes *Adenocalymma* Mart. ex Meisn., most species of *Memora* Miers, and two species of *Tanaecium* Sw. The other genera in the Pithecocteniinae (*Amphilophium*, *Glaziovia*, and *Haplolophium* [including *Urbanolophium*]) all had pollen of the same type: stephanocolpate (zonocolpate) with coarse reticulation (Gentry & Tomb, 1979: figs. 7, 8). This difference in pollen type, in addition to the calyx simple versus with a frilled margin, was used by Melchior (1927) as the first division in his key to genera of Pithecocteniinae to separate *Distictella* and *Pithecoctenium* (including *Neves-armondia*) from the other genera.

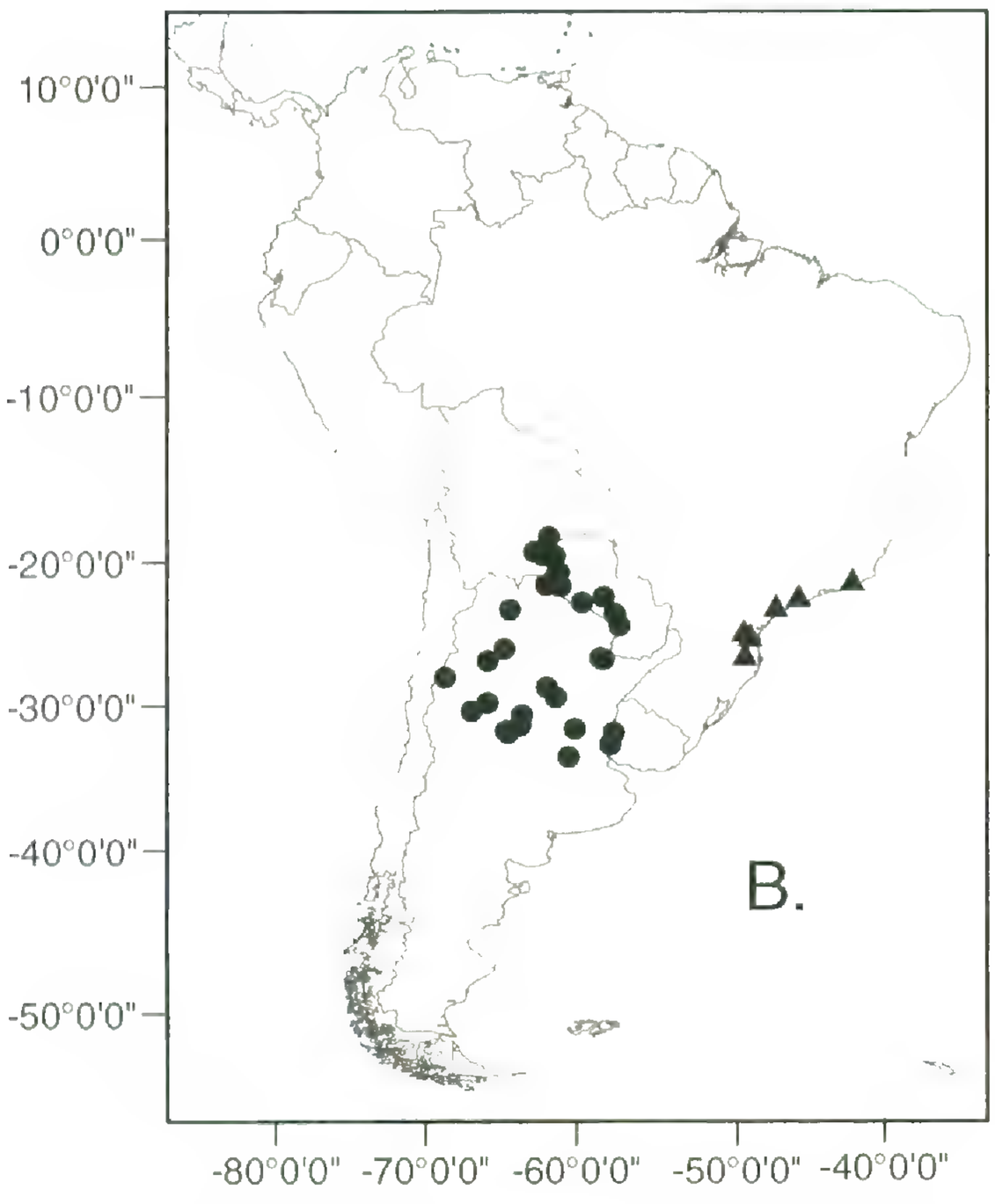
Distribution

Pithecoctenium is primarily a Neotropical genus, ranging from the Tropic of Cancer in Mexico to northern subtropical South America. *Pithecoctenium crucigerum* (Fig. 1A) ranges from the Tropic of Cancer in Mexico throughout Central America and into western South America through Peru and Bolivia to northern Argentina, into eastern South America through both Amazonian and Atlantic Brazil to Paraguay, northern Argentina, and Uruguay, and is also found in Cuba, Jamaica, and Trinidad, at 0–1900 m elevation, in any disturbed habitat. It is reported to naturalize in southern Florida (*Fisher s.n.*, FTG). Gentry (1973b) regards this as both geographically and ecologically one of the most wide-ranging species of Bignoniaceae. *Pithecoctenium carolinae* (Lindl.) G. Nicholson (Fig. 1B) is found in southeastern Bolivia, western Uruguay, northwestern Paraguay, and northern Argentina, at 50–1500 m elevation. It is generally reported to be found in dry forests, thorn scrub, or pasture. *Pithecoctenium dolichoides* (Fig. 1B) and *P. falcatum* (Fig. 1C) are from Atlantic Brazil, with one collection of *P. falcatum* from Bolivia. *Pithecoctenium dolichoides* is found below 1000 m elevation (and the habitat has not been described), and *P. falcatum* is found below 500 m, in disturbed areas.

Economic Uses

Species of *Pithecoctenium* are reportedly cultivated as ornamentals for their attractive flowers and interesting fruits. I have seen no report of *P. falcatum* being cultivated, and the use of *P. dolichoides* is probably restricted to Brazil (*F. C. Hoehne 33259*, NY). *Pithecoctenium crucigerum* and *P. carolinae* are cultivated sparingly in the southern United States (Menninger, 1970) (*N. F. Bracelin* 1430 [L], *J. B. McFarlin 5192* [MICH], respectively); cultivated specimens of both have been seen from southern tropical Africa (*H. M. Biegel 3423a* [MO], *H. M. Biegel 5529* [MO], respectively), and cultivated specimens of *P. crucigerum* have been seen from the Canary Islands (*G. Kunkel 11006*, G). *Pithecoctenium carolinae* is reportedly cultivated in Java (van Steenis, 1977).





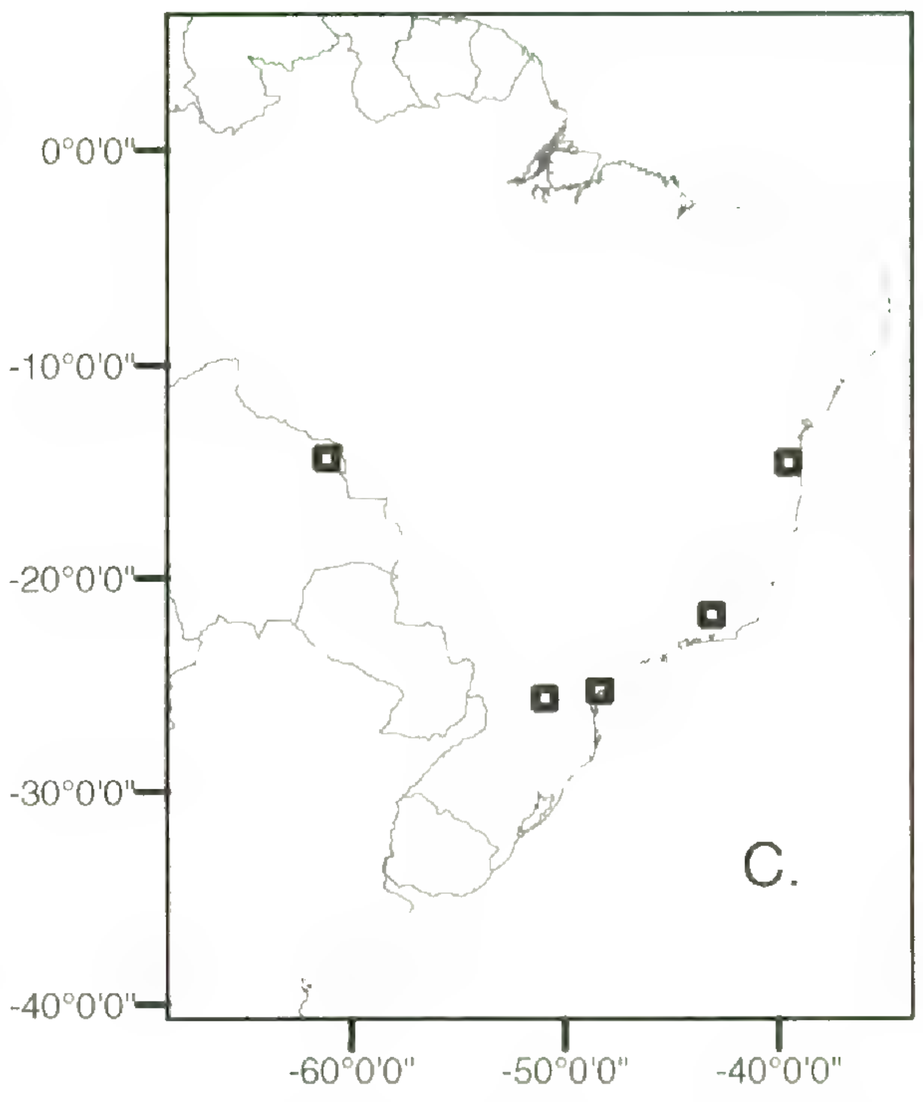


Figure 1. Distribution of *Pithecoctenium*, excluding cultivated and probably naturalized specimens. —A. *P. crucigerum* (L.) A. H. Gentry (■). —B. *P. carolinae* (Lindl.) G. Nicholson (●) and *P. dolichoides* (Cham.) Bureau ex K. Schum. (▲). —C. *P. falcatum* (Vell.) A. Pool (◘). Dots on the map represent a subset of the specimens examined by this author, including specimens with geographic coordinates provided on the collection labels and a set of specimens selected to illustrate the full geographic range for each species, for which the author estimated the geographic coordinates.

Materials and Methods

All specimens examined by Gentry and as part of this study have been incorporated into the Missouri Botanical Garden database-management system TROPICOS (http://mobot.mobot.org/W3T/Search/vast.html), which now contains label information for all *Pithecoctenium* specimens housed at MO in addition to those received on loan from AAU, B, BM, BR, C, CAS, CGE, CM, F, FTG, G, GH, GOET, L, M, MICH, NY, S, TEX, U, UPS, US, and W. Specimens determined by this author were selected from this database to generate the Index to numbered exsiccatae (Appendix 1) and the distribution maps, the latter with the assistance of Duan Bills. Dots on the maps (Fig. 1) represent a subset of the specimens examined by this author, including specimens with geographic coordinates provided on collection labels and a set of specimens, for which the author estimated the geographic coordinates, selected to illustrate the full geographic range for each species.

Taxonomic Treatment

Pithecoctenium Mart. ex Meisn., Pl. Vasc. Gen. 1: 300, 2: 208. 1840. TYPE: *Pithecoctenium echinatum* (Jacq.) Baill. [Based on *Bignonia echinata* Jacq.] (lectotype, designated by Sandwith, 1962: 454).

Neves-armondia K. Schum. in Engl., Nat. Pflanzenfam. Nachtr. 1: 301, 302. 1897. TYPE: *Neves-armondia cordifolia* K. Schum.

Lianas; branchlets acutely hexagonal with detachable ribs, without interpetiolar glandular fields; pseudostipules caducous. Leaves 2(3)-foliolate, if 2-foliolate usually with a tendril; tendril terminal, filiform, apically trifid with each branch usually additionally divided numerous times, some of the tips frequently thickened and disk-like. Inflorescence a raceme or racemose panicle; bracteoles inconspicuous, attached along length of pedicel, caducous. Flowers with the calyx campanulate, coriaceous, truncate, the ribs extending apically to form 5 small denticles, these sometimes inconspicuous; corolla tubular-campanulate, coriaceous, white, externally densely tomentose or sericeous above the tubular, particularly thickened, glabrous base, tube often bent at a right angle at a point 1/3 to 1/2 length above base, internally glabrous except for ridge where stamens and staminode are adnate; lobes broad, imbricate, internally tomentose; stamens 4, included, anthers glabrous, dithecal, the thecae straight, divaricate, staminode present, small; disc annular-pulvinate; ovary broadly elliptic or oblong, constricted above the disc, usually densely pubescent with appressed trichomes (glabrous in *P. falcatum*), bilocular, ovules multiseriate in each locule, style generally glabrous above base, stigma included, broadly ovate or rhombate. Capsule thick, somewhat compressed, oblong-elliptic, woody, the valves parallel to the septum, usually densely echinate or muricate (smooth in *P. falcatum*), the midrib not apparent; seeds in 2 or 3(4) rows, seeds with wing surrounding body on three sides and to varying degrees on side of attachment, wing shiny, hyaline-membranous, white or colorless with fine yellowish brown veins radiating from firmer zone around embryo, surrounding zone and embryo yellowish tan, smooth, minutely puberulent or papillose-puberulent.

When Meisner (1840) published the genus *Pithecoctenium*, he made no new combinations, but referred to de Candolle's unpublished *Prodromus* treatment (21 species) and cited published plates of *Bignonia echinata* Jacq. and *B. squalus* Vell. and text pages of *B. vitalba* Cham. and *B. botryoides* Cham. Martius (1841) published the first name in *Pithecoctenium*, *P. cordifolium* Mart., but made this name superfluous and illegitimate by citing *B. squalus* in synonymy. Sandwith (1962) treated *B. squalus* as synonym of *P. echinatum* (Jacq.) Baill. (based on *B. echinata* Jacq.) and chose this as the lectotype of the genus, stating "This is the most common, widely distributed and variable species of the genus, as well as the earliest named, and clearly it should be the lectotype, as I now propose" (Sandwith, 1962: 454). An earlier name for *P. echinatum* became available when Gentry (1975a) and Barrie (1991) lectotypified *B. crucigera* L. (1753), and the lectotype of the genus, *P. echinatum*, is treated here as a synonym of *P. crucigerum* (L.) A. H. Gentry.

Schumann (1897) published the monotypic genus, *Neves-armondia*, separating it from *Pithecoctenium* based on its glabrous ovary and smooth fruit. Melchior (1927) used these same characters to separate *Neves-armondia* from *Pithecoctenium*. Gentry (1979) returned *N. cordifolia* to *Pithecoctenium* (as *P. hatschbachii*) based on similar flower (excluding the glabrous ovary of *Neves-armondia*) and vegetative characters and identical pollen type (Gentry & Tomb, 1979). This placement is followed here and is further supported by the capsule of *N. cordifolia* [*P. falcatum*], which, in its overall appearance, except for the surface, is similar to that of *P. crucigerum*, and by its seeds, which are also very similar. Furthermore, as both smooth and echinate or tuberculate fruits are found within the same genus in other genera in the Pithecocteniinae (*Amphilophium* and *Haplolophium*), this difference does not seem worthy of generic recognition.

KEY TO SPECIES OF *PITHECOCTENIUM*

1. Ovary glabrous; fruit smooth or with very few dull tubercles; inflorescence usually a racemose panicle, or less frequently a raceme; 10–15 mm at base of seed free from seed wing; Brazil and Bolivia . . . . . 4. *P. falcatum*
1′. Ovary densely sericeous; fruit distinctly muricate or echinate; inflorescence a raceme or rarely a racemose panicle; up to 8 mm at base of seed free from seed wing 2
   2. Branchlets, inflorescences, and leaves villous with dense golden trichomes over 1.5 mm long; corolla 6.2–7 cm long, filaments of longer stamens 27–35 mm long; endemic to southern Atlantic Brazil . . . . . 3. *P. dolichoides*
   2′. Branchlets, inflorescences, and leaves variously puberulent, tomentose, pilose or glabrous, trichomes, when present, white, less than 1 mm long; corolla 2.5–6.8 cm long; filaments of longer stamens 12–25 (rarely 28) mm long; widely distributed in Neotropics 3
      3. Leaflets ovate to suborbicular with acute or briefly acuminate apices; capsules 8–31.5 cm long, 1.5–4.5 cm in diam., wall 1.5–4.5 mm thick, spines 1–7 mm long; 3–8 mm at base of seed free from seed wing; filaments of longer stamens 15–25 (rarely 28) mm long; Mexico to northern Argentina . . . . 1. *P. crucigerum*
      3′. Leaflets deltate with long-acuminate or long-caudate apices; capsules 4–13.5 cm long, 0.2–0.8 cm in diam., wall up to 1.5 mm thick, tubercles up to 1 mm long; up to 1.5 mm at base of seed free from seed wing; filaments of longer stamens 12–16 mm long; Bolivia, Uruguay, Paraguay, and Argentina . . . . . 2. *P. carolinae*

1. Pithecoctenium crucigerum (L.) A. H. Gentry, Taxon 24: 123. 1975. *Bignonia crucigera* L., Sp. Pl. 624. 1753. TYPE: Pt. 3, section 15, t. 3, f. 16 "PseudoApocynum folliculis maximis obtusis" in Morison, Pl. Hist. Univ. 1699 (lectotype, designated by Barrie, 1991: 265, t. 3, f. 16 "PseudoApocynum folliculis maximis obtusis" in Morison, 1699!).

Bignonia echinata Jacq., Enum. Syst. Pl. 25. 1760. *Pithecoctenium echinatum* (Jacq.) Baill., Hist. Pl. 10: 8, 37. 1891 [1888]. *Pithecoctenium echinatum* (Jacq.) K. Schum. in Engl., Nat. Pflanzenfam. 4(3b): 218. 1894, nom. illeg. TYPE: tab. 176, f. 52 in Jacq., Select. Stirp. Amer. Hist. 1763 (neotype, designated by Sandwith & Hunt, 1974: 154, tab. 76, f. 52 in Jacq., 1763!).

Bignonia tiliifolia Kunth in HBK, Nov. Gen. Sp. 3: 136. 1818 [1819] [as *tiliaefolia*]. TYPE: Venezuela. Orinoco [?], s.d., *F. Humboldt & A. Bonpland s.n.* (holotype, P not seen, photo F neg. 39417!)

Bignonia squalus Vell., Fl. Flumin. 244. 1825 [1829]. *Pithecoctenium squalus* (Vell.) DC. in A. DC., Prodr. 9: 194. 1845. *Pithecoctenium cordifolium* Mart., Flora 24(2, Beibl. 3): 48. 1841, nom. illeg. TYPE: tab. 13 in Vell., Fl. Flumin. Icones 6. 1827 [1831] (lectotype, designated here, tab. 13 in Vell., 1827 [1831]!).

Bignonia vitalba Cham., Linnaea 7: 699. 1832 [1833]. Syn. nov. *Bignonia vitalba* var. *aequinoctialis* Cham., Linnaea 7: 699. 1832 [1833]. *Pithecoctenium vitalba* (Cham.) DC. in A. DC., Prodr. 9: 196. 1845 [as *vitalbae*]. TYPE: Brazil. Equatorial, s. loc., s.d., *F. Sellow 3271* (lectotype, designated by Bureau & Schumann, 1896: 168, LE not seen; duplicate, B not seen, photo F neg. 18492!).

Bignonia vitalba var. *extratropica* Cham., Linnaea 7: 699. 1832 [1833]. Syn. nov. *Pithecoctenium vitalba* var. *extratropicum* (Cham.) DC. in A. DC., Prodr. 9: 196. 1845. TYPE: Brazil. Rio Grande do Sul: "prope fluvium Jacuhy et Rio Pardo," *F. Sellow 1515* (lectotype, designated by Bureau & Schumann, 1896: 168, LE not seen).

Bignonia botryoides Cham., Linnaea 7: 700. 1832 [1833]. Syn. nov. *Pithecoctenium botryoides* (Cham.) DC. in A. DC., Prodr. 9: 195. 1845. TYPE: Brazil. s. loc., s.d., *F. Sellow* (probably *1618(736)* from Minas Gerais, Sumidoro, Rio das Velhas) (holotype, LE not seen; isotype, B† not seen, photo F neg. 8478!).

Bignonia phaseoloides Cham., Linnaea 7: 698. 1832 [1833]. *Petastoma phaseoloides* (Cham.) Miers, Proc. Roy. Hort. Soc. London 3: 195. 1863. *Pithecoctenium phaseoloides* (Cham.) Schenck, Bot. Mitt. Tropen 4: 193. 1892. TYPE: Brazil. Santa Catarina: *A. Chamisso s.n.* (lectotype, designated by Sandwith & Hunt, 1974: 156, LE not seen).

Pithecoctenium aubletii Splitg., Tijdschr. Natuurl. Gesch. Physiol. 9: 12. 1842. TYPE: Surinam. s. loc., 26 Jan. 1838, *F. Splitgerber 544* (lectotype, designated by Sandwith, 1938: 46, L!).

Pithecoctenium catharinae DC. in A. DC., Prodr. 9: 196. 1845. Syn. nov. TYPE: Brazil. Santa Catarina: 1838, *C. Gaudichaud 179* (lectotype, designated by Sandwith & Hunt, 1974: 156, G-DC not seen, microfiche G-DC 9.196.12!).

Pithecoctenium hexagonum DC. in A. DC., Prodr. 9: 195. 1845. TYPE: Mexico. Chalco, s.d., *G. Andrieux 222* (holotype, G-DC not seen, photo F neg. 7654!; isotypes, K not seen, M!, W!).

Pithecoctenium hexagonum var. *brasilianum* DC. in A. DC., Prodr. 9: 195. 1845 [as var. *brasiliana*]. Syn. nov. TYPE: Brazil. Rio S. Francisco, 1838, *J. S. Blanchet 2905* (holotype, G-DC not seen, microfiche G-DC 9.195.8β!; duplicate, G!).

Pithecoctenium lundii DC. in A. DC., Prodr. 9: 196. 1845. TYPE: Brazil. São Paulo: Nov. 1835, *P. W. Lund 786* (lectotype, designated by Gentry, 1973b: 910 [as *876*], G-DC not seen, photo F neg. 7652!).

Pithecoctenium muricatum Moç. ex DC. in A. DC., Prodr. 9: 194. 1845. *Bignonia muricata* Sessé & Moç., Pl. Nov. Hisp. 99. 1889, nom. illeg. TYPE: Mexico. "agro Guatimalensi ubi vulgo Petachillos" [Morelos, Cuernavaca], tab. 820 in A. DC., Calques Fl. Mexique. 1874 (lectotype, designated by de Candolle, 1874: intro. [1], 4: tab. 820, tab. 820 in A. DC., 1874!).

Pithecoctenium echinatum var. *tomentosum* Hassl., Bull. Herb. Boissier 6(4, App. 1): 27. 1898. Syn. nov. TYPE: Paraguay. "Ad margines silvarum Cordillera de Altos,"

Jan., *E. Hassler 1766* (lectotype, designated here, G!; duplicate, NY!).

Pithecoctenium echinatum var. *intermedium* Hassl., Bull. Herb. Boissier 6(4, App. 1): 27. 1898. Syn. nov. *Pithecoctenium vitalba* var. *rostratum* Hassl., Repert. Spec. Nov. Regni Veg. 9: 56. 1910, nom. illeg. TYPE: Paraguay. "Central Paraguay," 1888–1890, *T. Morong 749* (holotype, G not seen; isotypes, F!, MICH!, MO!, NY!, US!).

Pithecoctenium echinatum var. *tomentosum* f. *grandiflorum* Hassl., Repert. Spec. Nov. Regni Veg. 9: 56. 1910 [as f. *grandiflora*]. Syn. nov. TYPE: Paraguay. "In campis rupestribus Cordillera de Altos," Dec., *E. Hassler 2127* (holotype, G not seen; isotypes, BM!, G!, GH!, NY!. W!).

Pithecoctenium vitalba f. *glabratum* Hassl., Repert. Spec. Nov. Regni Veg. 9: 56. 1910. Syn. nov. TYPE: Paraguay. "In dumetis ad ripas rivulorum pr. Esperanza," Nov., *T. Rojas [Hassler number series] 10708* (lectotype, designated here, G!; duplicates, A!, BM!. NY!, P not seen, S!, W!).

Pithecoctenium vitalba f. *intermedium* Hassl., Repert. Spec. Nov. Regni Veg. 9: 56. 1910. Syn. nov. TYPE: Paraguay. "In valle fl. Y-aca, in dumeto prope Chololo," Dec., *E. Hassler 6889* (lectotype, designated here, G!; duplicate, NY!).

Pithecoctenium glaucum Rusby, Mem. New York Bot. Gard. 7: 354. 1927. TYPE: Bolivia. Rurrenabaque, 1000 ft., 5 Jan. 1922, *M. Cárdenas 2063* (holotype, NY!; isotypes, K not seen, US!).

Young branchlets densely to sparsely tomentose or puberulent with ascending trichomes, or sparsely pilose, with trichomes less than 1 mm long, to glabrous or trichomes only at nodes, scattered to dense lepidote, without noticeable lenticels, interpetiolar ridges weak or absent; pseudostipules lanceolate, oblong or linear (rarely falcate or oblanceolate), 3–15 × 1–6 mm. Leaves with tendrils trifid, or more frequently additionally branched, tips usually filamentous, infrequently disk-like; petiole 2–11 cm long, tomentose or adaxially tomentose, dense to scattered short-pilose, puberulent, or glabrous; petiolule 0.5–7.5 cm long, tomentose or adaxially tomentose, dense to scattered short-pilose, puberulent, or glabrous; leaflets ovate to suborbicular, (rarely 3–) 4.5–17 × 2.2–12 cm, membranous, veins palmate at base, with 3 to 5 (rarely 6) pairs of secondary lateral veins, tomentose or short-pilose to glabrous or with trichomes restricted to base or veins, pellucid-lepidote, generally with large glands near axils of basal veins, base truncate to cordate or obtuse, apex acute, or obtuse or acute and then abruptly short-acuminate. Raceme, or rarely racemose panicle, 3–27 cm long with 3 to 30 flowers, peduncle, rachis, and pedicel tomentose (rarely puberulent) or peduncle and rachis pilose to glabrous and pedicel tomentose, lepidote; bracteoles lanceolate, narrow-elliptic, or linear, 2–4(rarely 8) mm long, caducous. Flower with calyx 7–17 × 8–15 mm, densely to sparsely tomentose, lepidote, often with large glands, especially near apex; corolla densely tomentose above base, white or often yellow in throat and adaxial surface of lobes, (rarely 3)3.5–6.8 cm long, tube (rarely 2)2.7–5.6 cm long, length of thickened glabrous section 5–16 mm, width of tube at mouth 12–25 mm, glabrous internally except with a villous ring of trichomes at stamen insertion, the trichomes turgid and moniliform, lobes 7–15 × 7–16(rarely 22) mm; stamens and staminode inserted 5–16 mm from base of tube, longer filaments (rarely 15–)17–25(rarely 28) mm long, shorter filaments 12–19(rarely 20) mm long, anthers 3–6 mm long, staminode 2–11 mm long; disc 1–3 × 3–7 mm; pistil 2.7–5.4 cm long, ovary broadly elliptic, 3–7 × 2–5 mm, densely sericeous, stigma broadly ovate or rhombate. Capsule 8–31.5 × 3.5–8 cm, 1.5–4.5 cm in diam., wall 1.5–4.5 mm thick, base acute to acuminate (rarely obtuse), apex rounded to acute, with echinate appendage remaining attached to replum and septum when valves fall away, valve surface echinate, the spines 1–7 × 1–3 mm, conical at base, the slender tips eventually breaking off with age, septum margin (rarely 0.4)0.6–1.5 cm thick at thickest point, funicular scars broadly oblong (rarely linear); seeds 2–4 × 3.5–9 cm, basal 3–8 mm free of wing.

Figures: Bureau and Schumann (1897: t. 86, as *Pithecoctenium echinatum*); Fabris (1965: fig. 19, i–k, as *Pithecoctenium echinatum*); Gentry (1973b: fig. 27, as *Pithecoctenium echinatum*).

Distribution. Mexico from the Tropic of Cancer throughout Central America and into western South America through Peru and Bolivia to northern Argentina, and into eastern South America through both Amazonian and Atlantic Brazil to Paraguay, northern Argentina, and Uruguay, also in Cuba, Jamaica, and Trinidad, at 0–1900 m elevation, in any disturbed habitat (Fig. 1A). Reportedly naturalizing in southern Florida (*Fisher s.n.*, FTG). Gentry (1973b) regarded this as both geographically and ecologically one of the most wide-ranging species of Bignoniaceae, although never extremely abundant. He suggested that its vast distribution is in large part due to the dispersability of the seeds, and poetically expressed "The lightness and aerodynamic stability of a floating seed of this species makes its path of descent a thing of beauty even in perfectly still air; with a breeze a seed remains airborne indefinitely" (Gentry, 1973b: 912–913).

Phenology. For the whole geographic range, flowering and fruiting throughout year. In Mexico, Central America, Colombia, and Venezuela, flowering primarily from April through August; in Peru and Amazonian and northern Atlantic Brazil, flowering

primarily from December to March; and in southern Atlantic Brazil, Bolivia, Paraguay, Argentina, and Uruguay, flowering primarily October to February. Gentry (1973b) reported that most flowering occurs (in Panama) during the wet season, but that individual plants may be found in flower at other times, and that the fruit takes almost a year to mature with the seeds released in the middle of the dry season.

Common names. Peine de mico (Mexico, Gentry, 1982b; Nicaragua, Gentry, 2001; El Salvador and a generalized Central America, Standley & Williams, 1974); Peine de mono (Panama, Gentry, 1973b; Venezuela, Gentry, 1982a; Mexico, Gentry, 1982b); Pente de macaco (Brazil, Sandwith & Hunt, 1974); Cucharilla (Ecuador, Dodson & Gentry, 1978); Rasqueta (Venezuela, Gentry, 1982a); Xache'ma'ax (Maya, Gentry, 1982b); Kees-keesie-kam-kam, Kapellendoos (Suriname, Sandwith, 1938); Espiguilla (Peru, Macbride, 1961); Barbasco (Bolivia, *Nee 35127*); Kaí Kiguá (Paraguay, *Schinini 5024*), Ka'i platillo (Paraguay, *Binacional 1119*); Cucharo, Cucharillo, Lengua de vaca, Petaquillas (seeds), Shape (Guatemala, Standley & Williams, 1974); Pico de pato, Bateíta (Central America, Standley & Williams, 1974); Xachextabay, Netoloc, Xtabay (Yucatan-Maya, Standley & Williams, 1974); Mariposas (Central America, seeds), Palomitas (Central America seeds) (Burger & Gentry, 2000).

Uses. Cultivated as an ornamental (Sandwith & Hunt, 1974); valves reported to be used for scouring and cleaning, or as a grater, leaves used in making lotion for skin problems (Standley & Williams, 1974). One report from Bolivia of whole vine being used to kill fish (*Nee 35127*).

Prior to 1975, most 20th-century botanists (Sandwith, 1938, 1945b; Macbride, 1961; Fabris, 1965; Adams, 1972; Gentry, 1973b; Sandwith & Hunt, 1974; Standley & Williams, 1974) treated this species as *Pithecoctenium echinatum* (Jacq.) K. Schum., based on *Bignonia echinata* Jacq. *Bignonia echinata* was published by Jacquin (1760) with a brief diagnosis and no indication of type. In 1763, Jacquin published a much more complete description of *B. echinata*, indicated the habitat as Carthagena (Colombia), and presented an excellent illustration of the capsule and seed. In 1974, Sandwith and Hunt proposed this illustration (Jacquin, 1763, tab. 76, f. 52) as the neotype of *B. echinata*. K. Schumann (1894) is generally credited with the transfer of *B. echinata* to *Pithecoctenium*, but the transfer had been previously made in 1888 by Baillon (1891).

In 1975, Gentry (1975a) selected *Miller s.n.* (BM) [= *Pithecoctenium echinatum*] as the lectotype of *Bignonia crucigera* L. and published the new combination *P. crucigerum* (L.) A. H. Gentry, a name with higher priority than *P. echinatum*. Barrie (1991) argued that the *Miller* specimen could not be selected as the lectotype as it is not clear that it was ever seen by Linnaeus. Barrie (1991) noted that, while there are no specimens in any of the Linnaean herbaria or in the Clifford herbarium (BM) with this name, there are three elements associated with it. Linnaeus (1753) included in his original publication the citations *Gron. virg.* 73 [Gronovius's *Flora Virginia* (1739, 1: 73)] and *Moris. hist.* 3. p. 672 s. 15. t. 3. f. 16 [Morison's *Plantae Historiae* (1699, 3: 672 [612])], and there is a specimen cited by Gronovius (*Clayton 100*, BM), and a plate in Morison (s. 15. t. 3. f. 16) (Barrie, 1991). In addition, Barrie (1991) presented evidence that Linnaeus had seen, prior to 1753, unpublished plates of Plumier, one of which Linnaeus annotated, with minor rephrasing, with his original 1753 description (Barrie, 1991). Gentry (1975a) rejected *Clayton 100* as the lectotype of *B. crucigera* as he found it to be a sample of *B. capreolata* L., a species that differs from the original description of Linnaeus for *B. crucigera*. He rejected the Plumier plate (which represents *Tanaecium crucigerum* Seem.), believing that it was not original material (Gentry, 1975a). The Morison plate and the *Miller* specimen both are of the same species, formerly referred to as *P. echinatum*, and Gentry (1975a) chose the specimen over the plate as the lectotype of *B. crucigerum*. Barrie (1991), finding the *Miller* specimen could not be used, selected the Morison plate, thereby preserving the current use of the name, *P. crucigerum*, in the sense of Gentry. While the Plumier plate could have been chosen as the lectotype, there is no reason to reject Barrie's (1991) selection.

Type materials of *Bignonia tiliifolia* Kunth, *Pithecoctenium aubletii* Splitg., *P. glaucum* Rusby, *P. hexagonum* DC., *P. lundii* DC., *P. muricatum* Moç. ex DC., and *B. squalus* Vell. were examined in this study, and, based upon these observations, the species were placed in synonymy of *P. crucigerum*, thereby following Gentry (1982a). The Vellozo plate 13 (1827) was chosen as the lectotype of *B. squalus* Vell., as no Vellozo *Bignonia* specimens have been found (Gentry, 1975b). Martius (1841) published *P. cordifolium* Mart. as a superfluous name for *B. squalus*, citing, in addition to the Vellozo plate of *B. squalus*, the specimen *Martius Herbarium Florae Brasiliensis 516* [*A. L. P. da Silva Manso s.n.*]. This specimen has been treated in the literature (Gentry, 1982a) as the type of *P. cordifolium*. However, as Martius (1841) did not specify the type of *P. cordifolium*, the type is automatically the type of *B. squalus* (International Code of Botanical Nomenclature 7.5 in McNeill et al., 2006). The lectotype of *P. muricatum* (de Candolle, 1874: t.

820) is a tracing of t. 217 in Sessé & Moç., Fl. Mex. Icones (McVaugh, 2000). *Bignonia muricata* Sessé & Moç. (Pl. Nov. Hisp. 99. 1889) cited this same plate and is, therefore, a superfluous name for *P. muricatum*.

The only material that de Candolle (1845) cited for *Pithecoctenium hexagonum* var. *brasilianum* DC. was a collection made by Blanchet in Brazil along the Rio São Francisco. The collection number, *2905*, is added here to the type citation and the name placed in synonymy of *P. crucigerum* based on observation of the G-DC microfiche of *Blanchet 2905* and the duplicate of *Blanchet 2905* at G.

Sandwith and Hunt (1974: 156) lectotypified *Bignonia phaseoloides* Cham. and treated this name as a synonym of *Pithecoctenium echinatum*, as did Bureau and Schumann (1896), who cited *F. Sellow 666*, a probable syntype or isosyntype. Gentry (1982b) treated *B. phaseoloides* as a synonym of *P. crucigerum*, based on Bureau and Schumann (1896), without seeing type material. Type material was not seen in this study, and *B. phaseoloides* is placed in synonymy strictly based on these earlier works.

Loesener (1919) published *Pithecoctenium tribrachiatum* Loes. with two syntypes, *J. H. Schenck 860* and *861*, both presumed at B and destroyed. The absence or existence of duplicates of the syntypes has not been confirmed in this study. Based on Loesener's (1919) description, in particular the shape of the branchlets and nature of the multi-trifurcate tendrils, Gentry's (1982a) treatment of this entity from Veracuz, Mexico, as a synonym of *P. crucigerum* is supported by this study.

Pithecoctenium botryoides (Cham.) DC. is placed in synonymy of *P. crucigerum* with some uncertainty. Chamisso (1832: 700) cited the type as "Brasilia. Sellow," a poor specimen with fallen flowers. Bureau and Schumann (1896: 170) cited two specimens: *Sellow 1618(736)* from Minas Gerais, Sumidoro, Rio das Velhas, and *Glaziou 14113* without locality. A photo (F neg. 8478) of *Sellow 1618(736)* (B) was seen in this study. Bureau and Schumann (1896) distinguished *P. botryoides* from *P. vitalba* and *P. echinatum* [= *P. crucigerum*] by its abbreviated inflorescence. It seems no different from other specimens of *P. crucigerum* with similar few-flowered racemes (such as *Irwin et al. 10343*, MO).

Examination of type materials of *Pithecoctenium catharinae* DC. and *Bignonia vitalba* Cham. leads to the names being placed in synonymy of *P. crucigerum*, following Sandwith and Hunt (1974), who treated these as synonyms of *P. echinatum* [= *P. crucigerum*].

Inflorescence types and a pubescent versus glabrous style base have been used to distinguish *Pithecoctenium vitalba* from *P. echinatum*. Hassler (1910) associated a racemose panicle and a pilose style base with *P. vitalba* and a raceme and glabrous style base with *P. echinatum*, while Bureau and Schumann (1896) associated a racemose inflorescence (in the key) and pilose style base with *P. vitalba* and a paniculate inflorescence (in the key) and glabrous style base with *P. echinatum*. While placing *P. vitalba* in synonymy with *P. echinatum*, Sandwith and Hunt (1974) found that specimens from southern Brazil and Paraguay had branched inflorescences and tended to have pubescent style bases, and suggested that these characters be carefully examined in studying *P. echinatum* and related entities. Interesting variation in these characters was noted in the course of this study. Branched inflorescences were only found in material collected in southern Brazil (Minas Gerais, Paraná, Santa Catarina, and Rio Grande do Sul), southeastern Paraguay (Caaguazú and Alto Paraná), and northern Argentina (Misiones); all of these (with flowers dissected) were found to have pilose style bases. However, pilose style bases were also found in material with unbranched inflorescences. One collection from Ceará (northeastern Brazil, *Gentry et al. 50193*, MO) had a pilose style base. All specimens (with flowers dissected) from Argentina with unbranched inflorescences had pilose style bases, while one collection from Rio Grande do Sul (southern Brazil), with an unbranched inflorescence, had a glabrous style base (*Pedersen 12620*, MO), and one had a pubescent style base (*Richter s.n.*, *HB-29882*, MO). All collections (with flowers dissected) from Central America, Cuba, Venezuela, Bolivia, and Amazonian Brazil had glabrous styles, while one collection from Colombia (*Smith 350*, MO) and one from Peru (*Núñez 9952*, MO) had pilose style bases. Flowers of the other three species in the genus were also examined: *P. dolichoides* was found to always have a pilose style base while that of *P. falcatum* was always glabrous (with a few lepidote scales), but the styles in *P. carolinae* varied from totally glabrous, to pilose at base only, to pilose nearly to the apex. While the variation in these characters is interesting, it does not seem worthy of taxonomic recognition.

Chamisso (1832) published *Bignonia vitalba* with two varieties, *B. vitalba* var. α, *aequinoctialis* and var. β, *extratropica*. Chamisso (1832) described *B. vitalba* var. α, *aequinoctialis* as villous-pubescent, with broadly ovate, cordate leaflets and inflorescences sometimes branching, while var. β, *extratropica* was described as glabrous, except for the petiolules, with narrow ovate leaflets with rounded to truncate bases and inflorescences simple racemes. De Candolle (1845) transferred *B. vitalba* to *Pithecoctenium*, treating var. *aequinoctialis* as the typical variety and

recognizing the second variety as *P. vitalba* var. *extratropicum*, based on its lack of pubescence and narrow ovate leaflets with truncate to rounded bases. Bureau and Schumann (1896) designated lectotypes for *P. vitalba* and *P. vitalba* var. *extratropicum* and recognized the latter on the same characteristics as de Candolle (1845), but suggested the recognition was uncertain in such a variable and widespread species. The type of *P. vitalba* var. *extratropicum* was not seen in this study, but the descriptions of Chamisso (1832), de Candolle (1845), and Bureau and Schumann (1896) suggest that it fits well within *P. crucigerum*, which varies throughout its geographic range in pubescence and leaf base. For example, in south Atlantic Brazil, specimens with simple racemes had leaf bases obtuse, truncate, subcordate, and cordate and abaxial leaf surfaces glabrous to pubescent only on veins to short-pilose or tomentose overall. Therefore, *P. vitalba* var. *extratropicum* is treated in this study as a synonym of *P. crucigerum* without varietal recognition.

Hassler (1898) published *Pithecoctenium echinatum* var. *tomentosum* Hassl. and *P. echinatum* var. *intermedium* Hassl., distinguishing them from the typical variety based on pubescence and leaf base characters. Three syntypes of *P. echinatum* var. *tomentosum* were seen in this study, the most complete chosen as the lectotype, and the name placed in synonymy with *P. crucigerum*. Five isotypes of *P. echinatum* var. *intermedium* were seen in this study, and, based on their examination, *P. echinatum* var. *intermedium* was also placed in synonymy of *P. crucigerum*.

Hassler (1910) published *P. echinatum* var. *tomentosum* f. *grandiflorum* based on its larger flowers, with calyx 12 mm long and corolla 6 cm long. Corolla size in *P. crucigerum* varies gradually from 3 to 6.8 cm long, with most of the flowers measured falling between 4.2 and 6.2 cm. Calyces vary gradually from 7 to 17 mm long, with most of the flowers measuring between 8 and 14 mm. The type of *P. echinatum* var. *tomentosum* f. *grandiflorum* was examined, and in all aspects fits well within the variation of *P. crucigerum*, and the name is placed in synonymy.

Hassler (1910) recognized *Pithecoctenium echinatum* var. *intermedium* Hassl. as a variety of *P. vitalba*, publishing the superfluous name *P. vitalba* var. *rostratum* Hassl. with two forms, *P. vitalba* var. *rostratum* f. *glabratum* Hassl. and *P. vitalba* var. *rostratum* f. *intermedium* Hassl. The forms were distinguished on pubescence characters. Three syntypes of *P. vitalba* var. *rostratum* f. *glabratum* and two of *P. vitalba* var. *rostratum* f. *intermedium* were seen; those with the most isosyntypes were chosen as the lectotypes, and the names were placed in synonymy of *P. crucigerum*.

Selected specimens examined. BELIZE. **Cayo:** S of Millionario, *A. H. Gentry 7666* (MO); 1 mi. E of Georgeville on Western Hwy., *Gentry 7855* (MO). COSTA RICA. **Guanacaste:** Cantón de Liberia, P.N. Santa Rosa, Península de Santa Elena, *J. F. Morales 2928* (MO). EL SALVADOR. **Sonsonate:** s. loc., *E. A. Montalvo 3446* (MO). GUATEMALA. **Petén:** Tikal National Park, Tikal, *E. Contreras 170* (MO). HONDURAS. **Francisco Morazán:** Inmediaciones de la U.N.A.H., *J. Bourdeth 44* (MO). MEXICO. **Chiapas:** 18.5 mi. N of Arriaga on rd. 200, *R. H. Maxwell 266* (MO). **Jalisco:** Mpio. de Autlán, 3 km al S de Ahuacapán, *E. J. Lott et al. 445* (MO). **Oaxaca:** Mun. Santiago Lachiguiri, Distr. de Tehuantepec, 17.8 km al NO de Lachiviza, camino a Santiago Lachiguiri, *C. Torres R. & A. Campos V. 13915* (MO). **Querétaro:** La Cuesta, 3 km al S de Escanelilla, Mpio. Pinal de Amoles, *N. Fernández R. 1582* (MO). **Tamaulipas:** Vic. of Victoria, *E. Palmer 403* (MO). NICARAGUA. **Estelí:** Rd. from Estelí N to Regado, then W to San Juan de Limay, then N to Pueblo Nuevo, *D. Neill 1200* (MO). PANAMA. **Canal Zone:** Barro Colorado Island, *R. Foster 840* (MO).

CUBA. **Granma:** Rd. to Pico Turquino Trail, Sierra Maestra, Río Yara Valley, *A. H. Gentry 50935* (MO). JAMAICA. **St. Catherine:** 1.5 mi. SE of Sligoville, *G. R. Proctor 31733* (F). TRINIDAD. Penal Rock Rd., *N. L. Britton et al. 1985* (NY).

ARGENTINA. **Corrientes:** Dep. General Paz, Tacuaracuarendú, *T. M. Pedersen 11527* (MO). **Misiones:** Montecarlo, 26 km S of Eldorado, *S. A. Renvoize et al. 3262* (MO); Dep. Guaraní, 7 km al W del Ayo. Soberbio, *J. H. Hunziker et al. 10835* (MO); Dep. San Pedro, entre San Pedro y Puerto Piray, km 76, *F. O. Zuloaga et al. 2061* (MO). BOLIVIA. **Beni:** Prov. Ballivan, Espiritu en la zona de influencia del Río Yacuma, *S. G. Beck 2573* (MO). **La Paz:** Prov. Nor Yungas, 2 km S of San Pedro on rd. to Yolosa, *J. C. Solomon & M. Nee 12607* (MO). **Santa Cruz:** Prov. Andres Ibañez, 1 km N of center of Cotoca, *M. Nee 33550* (MO); Prov. Cordillera, Alto Parapetí, *R. de Michel 226* (MO). BRAZIL. **Acre:** Rio Brancho, Km 1 ao Km 23 da Rod. Rio Brancho–Porto Velho, *J. U. Santos et al. 84* (MO). **Ceará:** 2 km S of Farias Brito, CE-021, *A. H. Gentry et al. 50193* (MO). **Goiás:** Serra do Rio Preto, ca. 14 km E of Cabeceiras, *H. S. Irwin et al. 10343* (MO). **Mato Grosso do Sul:** Antonio João, descida p/Campestre, *G. Hatschbach & J. M. Silva 49108* (MO); Cuiabá, *Martius Herbarium Florae Brasiliensis 516* [*A. L. P. da Silva Manso s.n.*] (BM, G, L, MO, NY, W). **Minas Gerais:** Ituiutaba, Fuindas, *A. Macedo 2037* (MO). **Paraná:** Mun. Campo Largo, Retire Grande, *G. Hatschbach 41085* (MO); Mun. Curitiba, Uberaba de Baixo, *G. Hatschbach 10277* (MO). **Rio de Janeiro:** Mun. de Petrópolis, Araras, Vale das Videiras, *D. Sucre et al. 10598* (MO). **Rio Grande do Sul:** Caracol, *E. Richter s.n.(HB-29882)* (MO); Mun. Gravataí, Fonte das Nimfos on rd. to S. Francisco de Paula, *T. M. Pedersen 12620* (MO). **Santa Catarina:** Mun. Chapecó, Moinho São Domingo, N of Chapecó, *L. B. Smith & R. M. Klein 13258* (MO); Mun. Lajes, Indios, *A. Lourteig 2263* (MO). COLOMBIA. **Bolívar:** Mpio. de Achi, entre San Agustin y Villa Uribe, Serrania de San Lucas, *V. Cuadros H. 1617* (MO). **Chocó:** carretera Panamericana, entre el Río San Pablo y el Río Pató, *E. Forero et al. 5537* (MO). **Guajira:** Mun. Mingueo, pié de la Sierra Nevada de Santa Marta, *V. Cuadros H. 2008* (MO). **Magdalena:** Santa Marta, *H. H. Smith 350* (MO). ECUADOR. **Guayas:** Cerro Azul, Cordillera Chongon–Colonche, behind Cemento Nacional, 12 km

W of Guayaquil, Quebrada Canoa, *A. H. Gentry & C. Josse 72352* (MO). FRENCH GUIANA. Route montant au Rorota, *H. Jacquemin 1931* (MO). **Cayenne:** Au pied de la colline du Montabo, *G. Cremers 5837* (MO). PARAGUAY. "In dametis ad ripam rivuli Salado," *E. Hassler 1475* (syntype for *P. echinatum* var. *tomentosum*, G; isosyntype, NY); "L'Assomption," *B. Balansa 485* (syntype of *P. echinatum* var. *tomentosum*, G); "in dumetis ad ripas rivulorum pr. Esperanza," *T. Rojas [Hassler number series] 10708-a* (syntype of *P. vitalba* f. *glabratum*, G; isosyntypes, A, BM); "in valle fl. Y-aca in dumeto prope Chololo," *E. Hassler 6673* (syntype of *P. vitalba* f. *glabratum*, G); "in dumetis pr. Villa Rica," *E. Hassler 8636* (syntype of *P. vitalba* f. *intermedium*, G). **Cordillera:** W side of Río Piribebuy basin, 27 km W of Arroyos y Esteros, *E. Zardini & C. Velázquez 20297* (MO). **Itapúa:** Reserva Salto Tembey, *D. R. Brunner 1310* (MO). PERU. **Cusco:** La Convención, Maranura, Chaullay, *P. Núñez 8123* (MO). **Junín:** Chanchamayo Province, Hacienda La Genoa, San Ramón, valley of Río Tulumayo, *A. H. Gentry et al. 73258* (MO). **Madre de Dios:** Tambopata Province, alrededores del Alberque turístico Cusco Amazónico, *P. Núñez et al. 9952* (MO); Tambopata Province, Las Piedra, Cusco Amazónico, *M. Timina & N. Jaramillo 2694* (MO). **San Martín:** N of Uchiza, *J. Schunke V. 5744* (MO). SURINAME. **Coronie:** Coronie, along rd. to Coppename Point, *A. M. E. Jonker-Verhoef & F. P. Jonker 569* (U). URUGUAY. **Cerro Largo:** Sierra de Ríos, *W. G. Herter 1893* (MO). VENEZUELA. **Barinas:** ca. 75 km SW of Barinas on hwy. to San Cristóbal, *A. H. Gentry & L. Puig-Ross 14354* (MO). **Guárico:** Ca. del Hato Becerra, sur del Río Orituco, carretera Calabozo–Cazorla, *G. Morillo et al. 1243* (MO). **Portuguesa:** 7 km S of Biscucuy, *A. H. Gentry & L. Puig-Ross 14369* (MO). **Zulia:** Dtto. Miranda, vía El Consejo–Quirós–El Pensado, ca. 5 km O de El Pensado, *G. S. Bunting 8668* (MO); Distrito Mara, vic. of Río Guasare, betw. Rancho 505 and Cerro Yolanda, *J. A. Steyermark et al. 122887* (MO).

2. Pithecoctenium carolinae (Lindl.) G. Nicholson, Ill. Dict. Gard. 3: 152. 1886. *Bignonia carolinae* Lindl., Edwards's Bot. Reg. 28: sub t. 45. 1842. TYPE: from cultivated material at Melbury, Lord Ilchester's seat in Dorsetshire, 1842, s. coll., s.n. (holotype, CGE!).

Pithecoctenium cynanchoides DC. in A. DC., Prodr. 9: 195. 1845. Syn. nov. TYPE: Argentina. Circa Buenos Aires, 1831, *C. H. Bacle 52* (holotype, G-DC not seen, microfiche G-DC 9:195.9!, photo F neg. 7657!; duplicate, G!).

Anemopaegma clematideum Griseb., Abh. Königl. Ges. Wiss. Göttingen 19: 222. 1874. Syn. nov. *Pithecoctenium clematideum* (Griseb.) Griseb., Symb. Fl. Argent. 257. 1879. TYPE: Argentina. Córdoba: s. loc., 1870, *P. G. Lorentz 120* (lectotype, designated by Fabris, 1965: 353, GOET!; duplicates, CORD not seen, GOET!).

Pithecoctenium cynanchoides var. *pellucidum* Hassl., Repert. Spec. Nov. Regni Veg. 9: 55. 1910. Syn. nov. TYPE: Paraguay. Gran Chaco, ad margines silvarum Loma Clavel, Nov., *E. Hassler 2630* (holotype, G not seen; isotype, BM!).

Young branchlets densely short-pilose, with trichomes ca. 0.2 mm long, to glabrous or with trichomes only at nodes, scattered-lepidote, without noticeable lenticels, interpetiolar ridges weak or absent; pseudostipules oblong (rarely spatulate), 2–9 × 0.5–2.2 mm. Leaves with tendrils trifid, or infrequently additionally branched, tips usually filamentous, infrequently disk-like; petiole 1.2–5 cm long, dense to scattered short-pilose, especially on the adaxial surface; petiolule 0.5–2.5 cm long, dense to scattered short-pilose, especially on the adaxial surface; leaflets deltate, 1.7–6.8 × 0.9–5 cm, membranous, veins palmate at base, with 3 to 5 pairs of secondary lateral veins, scattered pilose to glabrous or with trichomes restricted to base or veins, pellucid-lepidote, sometimes with large glands near axils of basal veins, base truncate to cordate, apex long-caudate or acute and then abruptly long-acuminate. Raceme 1.5–10 cm long with 2 to 14 flowers, peduncle, rachis, and pedicel densely short-pilose to glabrous or with trichomes restricted to nodes, lepidote; bracteoles narrow-elliptic, 2–10 mm long, caducous. Flowers with calyx 5–8(rarely 10) × 6–8 mm, densely to sparsely tomentose, lepidote, usually with large glands, especially near apex; corolla densely tomentose above base, white or often yellow in throat, 2.5–4.5(rarely 5.2) cm long, tube 1.8–3.7 cm long, length of thickened glabrous section 3–7(rarely 9) mm, width of tube at mouth 9–20 mm, glabrous internally except with a villous ring of trichomes at stamen insertion, the trichomes turgid and moniliform, lobes 6–15 × 5–18 mm; stamens and staminode inserted 4–8 mm from base of tube, longer filaments 12–16 mm long, shorter filaments 8–12 mm long, anthers 2.5–3 mm long, staminode 1.5–7 mm long; disc 1–1.5 × 2.2–3.3 mm; pistil 1.7–3.1 cm long, ovary broadly elliptic, 2–4 × 1.5–2.5 mm, densely sericeous, stigma broadly ovate or rhombate. Capsule 4–13.5 × 2–5 cm, 0.2–0.8 cm in diam., wall up to 1.5 mm thick, base obtuse to acute, apex obtuse to acute, with muricate appendage remaining attached to replum and septum when valves fall away, valve surface muricate, the tubercles 0.5–1 × 0.1–0.5 mm, conical at base, the slender tips generally breaking off with age, septum margin 0.1–0.3 cm thick at thickest point, funicular scars short and broad; seeds 1.5–3 × 2–4.2 cm, basal 0.5–1.5 mm free of wing, or thin marginal wing completely surrounding base.

Figures: Fabris (1965: fig. 19a–h, as *Pithecoctenium cynanchoides*); Fabris (1979: fig. 259, as *P. cynanchoides*); Lindley (1844: tab. 54, as *Bignonia carolinae*).

Distribution. Southeastern Bolivia, western Uruguay, northwestern to central Paraguay, and northern Argentina at 50–1500 m elevation. It is generally reported to be found in dry forests, thorn scrub, or pasture (Fig. 1B).

Phenology. Flowering September through March, primarily collected in flower in October through December; fruiting November through July.

Common names. Kalakalá (Paraguay, *Gragson 287*), Waka te le pep (Paraguay, *Arenas 2397*), Galleta de loco (Argentina, *Kiesling 4503*), Peine de mono (Argentina, *Novara 2148*; Fabris, 1965), Tripa de fraile (Argentina, Fabris, 1965), Yawxtii'táx (Argentina, *Arenas 2164*), Guitarrita, Pitito blanco, Loconte, Trompetilla de venus, Tripa de braya (Fabris, 1965).

Uses. The fruits are reportedly boiled or roasted over coals and the seeds eaten (Manxuj-Paraguay, *Gragson 287*). Cultivated as an ornamental (Fabris, 1965).

De Candolle (1845) based *Pithecoctenium cynanchoides* DC. on material that he had in his herbarium that had been collected by Bacle near Buenos Aires. The collection number *52* is added here to the type citation based on observation of the microfiche and photo of *Bacle 52* at G-DC. The duplicate of *Bacle 52* at G was also studied. This entity has been treated by most 19th- and 20th-century authors (Bureau & Schumann, 1896; Fabris, 1965, 1979) as *P. cynanchoides*. However, the type of *Bignonia carolinae* Lindl. was observed in this study and found to represent the same species as *P. cynanchoides*, a relationship that had been suggested by Miers (1863). *Pithecoctenium cynanchoides* is therefore placed in synonymy of *P. carolinae* (Lindl.) G. Nicholson (based on *B. carolinae*), a name with higher priority. The type of *B. carolinae* is a specimen at CGE taken from a plant grown at the estate of the Earl of Ilchester at Melbury in Dorsetshire. It is believed to have been originally collected in Buenos Aires (Lindley, 1844). A plate in Lindley (1844: t. 54) is reportedly drawn from material from the same plant from which the original specimen was obtained in 1842 (Lindley, 1844). The plate is fairly accurate, except for the tendrils, which are depicted as bifurcate. De Candolle apparently did not see the specimen of *B. carolinae* Lindl. and maintained the species in *Bignonia*, and the name was not included in either Bureau and Schumann (1896) or Fabris (1965, 1979).

Fabris (1965) designated the lectotype of *Anemopaegma clematideum* Griseb. and placed this into synonymy of *Pithecoctenium cynanchoides* [= *P. carolinae*]. The lectotype and one of its duplicates (at GOET) were seen in this study, and, based upon these observations, the species was placed in synonymy of *P. carolinae*.

Type material of *Pithecoctenium cynanchoides* var. *pellucidum* Hassl. was studied, and the variety was placed into synonymy of *P. carolinae*; the characteristics described by Hassler (1910), pellucid-lepidote leaves, long-caudate leaflets, and capsules 8 cm long and 3 cm wide, are consistent with typical *P. carolinae*.

Pithecoctenium carolinae is most easily distinguished from the closely related *P. crucigerum* using fruit characters: the former has a smaller, thinner fruit (4–13.5 × 2–5 cm, 0.2–0.8 cm in diam. vs. 8–31.5 × 3.5–8 cm, 1.5–4.5 cm in diam.), the valves of which have a thinner wall (up to 1.5 mm thick vs. 1.5–4.5 mm thick) and are covered with minute tubercles (0.5–1 × 0.1–0.5 mm) versus the more substantial spines (1–7 × 1–3 mm) found in *P. crucigerum*. Leaflet size has also been used to separate the two (Fabris, 1965), but *P. crucigerum* frequently has leaflets as small (4.5–7 × 2.2–6 cm) as *P. carolinae* with medium to large leaflets (4.5–6.8 × 3–5 cm). Leaflet shape is a more dependable character, but difficult to express: the leaflets of *P. carolinae* are deltate with long-acuminate apices, while those of *P. crucigerum* are ovate to suborbicular with briefly acuminate apices. The flowers of *P. carolinae* tend to be smaller than those of *P. crucigerum* in all aspects, but there is a good deal of overlap in size. For example, the calyx of *P. carolinae* is 5–10 mm long while that of *P. crucigerum* is 7–17 mm long, the corolla of *P. carolinae* is 2.5–5.2 cm long while that of *P. crucigerum* is 3–6.8 cm long, and the longer filaments of *P. carolinae* are 12–16 mm long while those of *P. crucigerum* are 15–28 mm long. *Pithecoctenium carolinae* generally has tendrils simply trifid, while in *P. crucigerum* the three branches of the tendril are commonly additionally branched. The geographic ranges of the species seem to overlap only in southern Bolivia and northernmost Argentina.

Selected specimens examined. ARGENTINA. **Chaco:** Dep. 12 de octubre, Gral. Pinedo, *A. G. Schulz 8936* (MO). **Corrientes:** 5 km S of Corrientes on Ruta 12, *A. H. Gentry et al. 59116* (MO); Dep. Empedrado, Estancia La Yela, *T. M. Pedersen 2972* (MO); Dep. Itatí, Ruta 12, desvío, a Itatí 6 km, *I. Schinini & L. Wroginski 1736* (MO). **Santiago del Estero:** Dep. Aguirre, Malbrán, *A. A. Saenz & G. Ruhl 199* (MO). **Tucumán:** Tafi Viejo, near Las Tipas, *S. Renvoize et al. 3381* (MO). BOLIVIA. **Chuquisaca:** Prov. Luis Calvo, Boyuibe 104 km hacia el este, via F. Villazón, *S. G. Beck & M. Liberman 9422* (MO). **Santa Cruz:** Prov. Corillera, 2–3 km NW of Boyuibe, along abandoned railroad to Cuevo, *M. Nee 35304* (MO). PARAGUAY. **Chaco:** Parque Nac. Defensores del Chaco, entre la casa forestal de Aguadulce y Aguairca, *J. Fernandez Casas & J. Molero 1375* (MO). **Nueva Asunción:** Km 695 de Asuncion, Chaco, *L. Bernardi 20242* (MO). **Presidente Hayes:** Fortín Tinfunqué, *R. Spichiger et al. 2070* (MO). URUGUAY. **Paysandú:** Paysandú, *W. G. Herter 1032* (MO).

3. Pithecoctenium dolichoides (Cham.) Bureau ex K. Schum. in Engl., Nat. Pflanzenfam. 4(3b): 218. 1894. *Bignonia dolichoides* Cham., Linnaea

7: 696. 1832 [1833]. TYPE: Brazil. "Brasilia meridionalis a S. Paulo ad meridiem," s.d., *F. Sellow s.n.* [herbarium no. *332*] (holotype, B†, photo F neg. 18484!; isotype, K not seen).

Young branchlets densely golden long-villous with trichomes over 1.5 mm long, scattered lepidote, without noticeable lenticels, interpetiolar ridges weak or absent; pseudostipules spatulate or ovate and basally attenuate, 3–20 × 2–15 mm. Leaves with tendrils trifid, or more frequently additionally branched, tips frequently disk-like; petiole 1–4.5 cm long, densely golden long-villous; petiolule 1–3 cm long, densely golden long-villous; leaflets ovate (rarely broadly oblong), 6–16 × 2.5–10.5 cm, membranous, veins palmate at base, with 4 to 7 pairs of secondary lateral veins, dense long-villous, pellucid-lepidote, base subcordate to deeply cordate, apex acute and abruptly short-acuminate or short-caudate. Raceme 13–25 cm long with 10 to 20 flowers, peduncle, rachis, and pedicel densely golden long-villous, lepidote; bracteoles filiform, 3–10 mm long, caducous. Flowers with calyx 7–13 × 10–13 mm, densely tomentose to sericeous, lepidote; corolla densely sericeous above base, white or cream to yellow, 6.2–7 cm long, tube 4.5–5.8 cm long, length of thickened glabrous section 8–10 mm, width of tube at mouth 15–25 mm, glabrous internally except with a villous ring of trichomes at stamen insertion, the trichomes turgid and moniliform, lobes 10–16 × 10 17 mm; stamens and staminode inserted 5–10 mm from base of tube, longer filaments 27–35 mm long, shorter filaments 20–21 mm long, anthers ca. 4 mm long, staminode 6–10 mm long; disc 1.5–3 × 3.5–6 mm; pistil 3.7–4.3 cm long, ovary broadly elliptic, ca. 6 × 4–4.2 mm, densely sericeous, stigma broadly rhombate or obovate. Mature capsule and seed not seen, immature capsule 11.5 × 3.5 cm, 0.7 cm in diam., base acute, apex acute, valve surface echinate, the spines ca. 2 × 2 mm, laterally flattened at base, the slender tips generally breaking off with age.

Distribution. Southern Atlantic Brazil, from Minas Gerais to Rio Grande do Sul (Sandwith & Hunt, 1974), Santa Catarina, São Paulo, Rio de Janeiro, and Paraná observed in this study, at 800 m elevation (Fig. 1B).

Phenology. Flowering October through January, immature fruits collected in January.

Common names. Pente de maco (Sandwith & Hunt, 1974; *W. Hoehne 1532*).

Uses. Cultivated as an ornamental (Sandwith & Hunt, 1974).

Chamisso (1832: 698) published *Bignonia dolichoides* Cham., citing the type as "in Brasilia intratropica legit Sellow." Sandwith and Hunt (1974) provided further collection locality details and a herbarium number, and indicated the deposition of the holotype at B and an isotype at K. An additional collection at US (labeled *Sellow 38*, digitized image at http://ravenel.si.edu/botany/types/!) is a possible isotype.

This species is distinguished from the widespread and variable *Pithecoctenium crucigerum* primarily based on the very distinctive long golden trichomes that are found on most of the vegetative parts as well as the inflorescence peduncle, rachis, and pedicels. While vegetative and inflorescence pubescence is a very variable character in *P. crucigerum*, no trichomes have been seen in that species that approach the length of those in *P. dolichoides* (longer than 1.5 mm). The flowers of *P. dolichoides* also tend to be somewhat larger (corolla 6.2–7 cm) than those of *P. crucigerum* (3–6.8 cm); however, there is a wide range in flower size in the latter and flower size ranges do overlap for the two species. It could be argued, as Sandwith and Hunt (1974) suggest, that *P. dolichoides* might be better treated as an infraspecies of *P. crucigerum.* Collection of the mature fruit and seed would help in determining the relationship of these two taxa.

Selected specimens examined. BRAZIL. **Paraná:** Mun. Balsa Nova, campina da Cascavel, *G. Hatschbach 39175* (MO); Mun. Tijucas do Sul, Pirai, *G. Hatschbach 40395* (MO); Mun. S. José dos Pinhais, Guaricana, *J. M. Silva & P. Ruas 80* (MO). **Rio de Janeiro:** Alto Macabé de Nova Fríburgo, *A. Glaziou 18379* (MO). **Santa Catarina:** Rio do Sul, Alto Matador, pinhal, *P. R. Reitz & R. M. Klein 7286* (NY). **São Paulo:** Apiraí, sítio da Pedra, *K. D. Barreto et al. 3137* (MO).

4. **Pithecoctenium falcatum** (Vell.) A. Pool, comb. nov. *Bignonia falcata* Vell., Fl. Flumin. 245. 1825 [1829]. TYPE: tab. 17 (in part) in Vell., Fl. Flumin. Icones 6. 1827 [1831], including branchlet, leaves, fruit, and seeds and excluding inflorescence and flowers (lectotype, designated here, tab. 17 (in part) in Vell., 1827 [1831]!). EPITYPE: Brazil. Bahia: Coaraci, Almadina, 18 km de Estrada, *T. S. dos Santos 574* (epitype, designated here, MO!; duplicates, CEPEC not seen, NY!). Figure 2.

Pithecoctenium cordifolium DC. in A. DC., Prodr. 9: 194. 1845, non *Pithecoctenium cordifolium* Mart., 1841. Syn. nov. *Neves-armondia cordifolia* K. Schum., nom. illeg. *Pithecoctenium hatschbachii* A. H. Gentry, Ann. Missouri Bot. Gard. 66: 784. 1979 [1980]. TYPE: Brazil. São Paulo: Prope Jacasava, Dec. 1817, *C. Martius 408* (lectotype, designated by Gentry, 1979: 784, M!).

Young branchlets glabrous, without noticeable lenticels, interpetiolar ridges present; pseudostipules not seen. Leaves with tendrils trifid, or additionally branched, tips filamentous; petiole 3.5–9 cm long,

Figure 2. *Pithecoctenium falcatum* (Vell.) A. Pool, excluding flowers. Reprinted from Vellozo, 1827 [1831], t. 17, as *Bignonia falcata* Vell.

glabrous; petiolule 2.5–6.5 cm long, glabrous; leaflets ovate to lanceolate, 8–15 × 6–12 cm, membranous, veins palmate at base, with 3 to 6 pairs of secondary lateral veins, glabrous or with trichomes restricted to base adaxially, pellucid-lepidote, with large glands near axils of basal veins, base deeply cordate, apex acuminate or acute, and short-caudate. Racemose panicle (or rarely raceme), 20–25 cm long with 25 to 28 flowers, peduncle, rachis, and pedicel puberulent or tomentose or peduncle glabrous, lepidote; bracteoles awl-shaped, ca. 5 mm long, caducous. Flowers with calyx 10–13 × 12–15 mm, tomentose at least at base, lepidote, sometimes with scattered large glands; corolla densely tomentose above base, white to cream or yellow in throat, 4.3–5.6 cm long, tube 3.3–4.5 cm long, length of thickened glabrous section 12–15 mm, width of tube at mouth 15–19 mm, glabrous internally except with a villous ring of trichomes at stamen insertion, the trichomes turgid and moniliform, lobes 10–12 × 10–15 mm; stamens and staminode inserted 10–12 mm from base of tube, longer filaments ca. 25 mm long, shorter filaments ca. 22 mm long, anthers 5–6 mm long, staminode 5–6 mm long; disc 2–3 × 4–7 mm; pistil 4.5–4.8 cm long, ovary oblong, 5–6 × 2–3 mm, lepidote and otherwise glabrous, stigma broadly elliptic or rhombate. Capsule ca. 28 × 5.5 cm, ca. 2 cm in diam., wall ca. 5 mm thick, base attenuate, apex acute without apical appendage, valve surface smooth with very few, small, dull tubercules, septum margin ca. 1.3 cm thick at thickest point, funicular scars long and linear; seeds 2.8–3 × 6–7.5 cm, basal 10–15 mm free of wing.

Figures: Vellozo (1827: t. 17, excluding inflorescence and flowers, as *Bignonia falcata*), reproduced here as Figure 2; Gomes (1952: pl. 6).

Distribution. Atlantic Brazil, specimens seen from Bahia (the epitype), Minas Gerais, and Paraná at 0–240 m elevation, and Velasco Province of Santa Cruz, Bolivia, at 150 m; also reported from Pernambuco (Bureau & Schumann, 1896) and Rio de Janeiro (Schumann, 1897) in Brazil. It is reported to be found in disturbed areas (Fig. 1C).

Phenology. Flowering December to February; fruiting July.

Vellozo published the name *Bignonia falcata* in 1829 with the following diagnosis: "*B.* folii conjugates, cirrhosis; foliolis cordatis, pedicellis trifloris; siliqua magna, falcate" (Vellozo, 1825: 245) and cited tableau 17 in volume 6 of his Icones series. The plate (Vellozo, 1827), reproduced here as Figure 2, depicts a branchlet with a (poorly drawn) racemose panicle with buds, mature flowers, and a mature fruit, as well as a dissected flower and seed. The vegetative characters: the strongly 6-angled stem, bifoliolate leaves with terminal trifid tendril, and ovate-cordate leaflets with basal palmate venation, as well as the shape and surface of the fruit and overall appearance of the seed, clearly represents the specimens here cited under *Pithecoctenium falcatum.* The flowers are not *Pithecoctenium.* The calyx is small and narrow, the corolla is straight, the stamens are exserted with sagittate anthers, the nectary disc is absent, and the ovary is not differentiated from the style. No Vellozo *Bignonia* specimens have been found (Gentry, 1975b), necessitating lectotypification of the Vellozo names based on the Vellozo Icones plates.

The name *Pithecoctenium cordifolium* was originally published by Martius in 1841 for a species described as having an echinate capsule. He cited a collection from Cujaba, Brazil (*Martius Herbarium Florae Brasiliensis 516*), but also placed *Bignonia squalus* Vell. in synonymy, making his name superfluous and illegitimate. De Candolle (1845) transferred *B. squalus* to *Pithecoctenium*, citing below *P. squalus* (Vell.) DC. "*P. cordifolium* ex parte Mart. herb." (de Candolle, 1845: 194), and recognizing another collection that Martius had made in São Paulo and identified as *P. cordifolium* as a separate species, which he named *P. cordifolium* DC., a later homonym of the validly published *P. cordifolium* Mart. Bureau and Schumann (1896) placed both *P. cordifolium* Mart. and *P. squalus* DC. in the synonymy of *P. echinatum*. They recognized *P. cordifolium* DC. (as *P. cordifolium* Mart.) as a separate species, which they placed in its own section, *Leiogyne*, based on its glabrous ovary (and pellucid punctate leaflets). Bureau and Schumann had no fruits of this species at this time, but noted both that the Vellozo plate of *B. falcata* depicted without doubt their recognized *P. cordifolium*, "Inter species *Bignoniae* a cl. Vellozo depictas unam invenimus quae fere sine dubitatione pro *Pithecoctenium cordifolio* habendaest, nempe *Bignonia falcata* Vell. Fl. Flum. VI t. 17" (Bureau & Schumann, 1896: 165) and, that if the capsule truly is smooth, the species should be moved to a separate and allied genus. Subsequent to this, Schumann did receive a fruiting specimen from Herrn Neves-Armond, and, in 1897, published the new monotypic genus for this species, *Neves-armondia*, separating it from *Pithecoctenium* based on its glabrous ovary and smooth fruit, and made the new combination *Neves-armondia cordifolia* K. Schum, a nom. nov. for *P. cordifolium* DC. (cited as *P. cordifolia* Mart.). At the same time, Schumann noted that *N. cordifolia* is the same as *B. falcata* illustrated by Vellozo, making *N. cordifolia* superfluous and illegitimate.

In 1975, Gentry (1975b) published an article attempting to account for the names of *Bignonia*

published by Vellozo (1825, 1827). In this, he stated (Gentry, 1975b: 342) "*Bignonia falcata* Vell. (245 (231) t. 17) is almost certainly a mixture based in part on *Neves-armondia cordifolia* (Mart. ex DC.) K. Schum. The vegetative parts and fruit are clearly those of the monotypic *Neves-armondia* but the illustrated flowers are completely different from the *Pithecoctenium*-like flowers of *N. cordifolia*. The probable identification of *B. falcata* with *N. cordifolia* was noted by Bureau and K. Schumann (1896–1897) and by K. Schumann (1897) and *B. falcata* has priority over the basionym of *N. cordifolia* (based on *Pithecoctenium cordifolium* Mart. ex DC., Prodr. 9: 194. 1845). However, in my opinion, the flowers illustrated for *B. falcata* are so dissimilar to those of *N. cordifolia* as to justify rejection of the former under Article 40 as based on a mixture of two or more elements." There is no provision in the 2006 version of the International Code of Botanical Nomenclature (McNeill et al., 2006) to allow for automatic rejection of *B. falcata* based on a "mixture of two or more elements," and there seems little justification to propose rejection of this name, which when lectotypified as above, presents no confusion. In addition, as the only other legitimate name published for this taxon, *P. hatschbachii* (published by Gentry, 1979, as a nom. nov. for *P. cordifolia* DC.), has been little used in the botanical literature, the lectotypification of *B. falcata* and new combination *P. falcatum* should cause little disturbance. A flowering specimen, *T. S. dos Santos 574* (MO, with duplicates at CEPEC, NY), is chosen as an epitype to clarify the lectotype, which excludes the flowering portion of t. 17 (Vellozo, 1827). The transfer of *B. falcata* to *Pithecoctenium* is based on its overall vegetative, floral (excluding the glabrous ovary), fruit (excluding surface), and seed similarity to *P. crucigerum*. In addition, Gentry and Tomb (1979) found its pollen to be identical to that of *P. crucigerum*.

Pithecoctenium falcatum is distinguished from *P. crucigerum* by its glabrous ovary and nearly smooth capsule. Racemose paniculate inflorescences and glabrous vegetative parts, while found in specimens of both species, are more commonly present in specimens of *P. falcatum*. However, sterile material, particularly less pubescent specimens, and flowering collections in which the flowers cannot be dissected, cannot be identified with confidence to species.

Additional specimens examined. BOLIVIA. **Santa Cruz:** Velasco Province, carretera al Chore, entre Arroyo de las Londras y Arroyo del Tigre, *M. Peña-Chocarro & L. Arroyo 154* (MO). BRAZIL. s. loc., *A. Glaziou 12972* (C), *11253* (C). **Minas Gerais:** Estação Experimental de Agua Limpa, *E. P. Heringer 2231* (NY). **Paraná:** Guaraqueçaba, 20–50 m, *G. Hatschbach 18505* (MO). **São Paulo:** s. loc., *C. Martius s.n.* (M, photo F neg. 20461).

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Appendix 1. Index to Exsiccatae.

Collections are alphabetical by collector name. After the collector's number, the number in parentheses corresponds to the species number in the List of Species. Type collections appear in boldface. All collections cited here were examined by this author as part of this study.

List of Species

1. *Pithecoctenium crucigerum* (L.) A. H. Gentry
2. *Pithecoctenium carolinae* (Lindl.) G. Nicholson
3. *Pithecoctenium dolichoides* (Cham.) Bureau ex K. Schum.
4. *Pithecoctenium falcatum* (Vell.) A. Pool

Acosta 16 (1); *Agra et al. 5013* (1); *Alboff s.n.* (2); *Alfaro 1816*(1); *Allen 6467* (1), *6579* (1); *Andrade-Lima 70-5781* (1); *Andersson s.n.* (1); *Andrieux* **222 (1)**; *Andrieux 108* (1); *Aplin s.n.* (1); *Araque M. et al. 19Ar437* (2), *19Ar447* (2); *Araquistain 2972* (1), *3704* (1); *Arbo 1555* (2); *Arbo et al. 6103* (1); *Archer 4736* (1); *Arechavaleta 20* (1); *Arenas 2164* (2), *2397* (2); *Aristeguieta et al. 6332* (1); *Atwood & Neill AN138* (1); *Ave-Lallemant s.n. (R23771)* (1).

Babcock 863 (1); *Bacle* **52 (2)**; *Bacle s.n.* (2); *Baer 15* (2); *Baker 11608* (1); *Balansa 484* (1), **485 (1)**, *3242* (1); *Balegno 15* (2), *656* (2), *1277* (2), *1402* (2); *Banks, s.n.* (1); *Bang 1668* (1); *Barkely 19Ar552* (2); *Barreto et al. 1822* (1), *3137* (3); *Bartlett 19568* (2), *19790* (2), *19834* (2), *19874* (2), *20090* (2), *20443* (2); *Basualdo & Zardini 1276* (2); *Beck 2573* (1), *5189* (1); *Beck & Liberman 9422* (2); *Belém 1916* (1); *Belém & Pinheiro 3108* (1); *Berlandier s.n.* (1); *Bernardi 18104* (1), *18675* (1), *18839* (1), *20180* (1), *20242* (2); *Berro 198*(2), *4870* (1); *Bertoni de Blanquis 2345* (1), *3443* (1); *Biegel 3423a* (1), *5529* (2); *Billiet & Jadin 3234b* (2); *Biolley 975pp* (1); *Blanchet 1061* (1), **2905 (1)**, *Blanchet s.n.* (1); *Böcher et al. 2458* (2); *Boffa s.n.* (2); *Boone 142* (1), *941* (1); *Bordas 4057* (1); *Bordas & Schmeda 4001* (1); *Bornmüller 613* (1); *Botta & Miconi 615* (2); *Boudet-Fernandez & Boone 1979* (1); *Bourdeth 44* (1); *Bourgeau 2462* (1), *Bourgeau s.n.* (1); *Bowie & Cunninham 59* (1); *Bracelin 1430* (1); *Bradley 294a* (1); *Breedlove 9823* (1), *51527* (1); *Breedlove & Raven 13657* (1); *Brenes 3990* (1), *4276* (1), *6168* (1), *6180* (1); *Britton et al. 1985* (1), *6680* (1); *Brizuela 354* (2), *406* (2), *475* (2), *775* (2), *867* (2); *Broadway 422* (1), *802* (1); *Brown 13376* (1); *Bruch 9603* (2), *Bruch s.n.* (2); *Brücher & Brücher s.n.* (2); *Brunner 1310* (1), *1584* (2); *Bueno et al. 19886* (1); *Bunting 4784* (1), *8668* (1); *Buratovich 949* (2); *Burger &*

Liesner 6700 (1); *Burkart 7443* (2), *10434* (2), *20643* (2), *22305* (2), *28881* (2); *Burkart & Troncoso 28035* (2); *Butner 371* (1); *Butzke et al. 7334* (1).

C.V.C. 1676 (1); *Cabrera 2595* (2), *16323* (2), *10563* (2), *12396* (2); *Cabrera & Kiesling 20333* (1); *Calderón 958* (1), *2179* (1); *Callejas & Hage 1973* (1); *Cárdenas* ***2063*** **(1)**, *2645* (2), *4174* (1); *Cascante & Vega 1000* (1); *Castillo C. & Tapia 751* (1); *Castillon 1004* (2), *56988* (2); *Chacón 695* (1); *Charpin & Eskuche 20093* (2); *Chavarría 627* (1), *1029* (1), *1251* (1); *Chavez A. 100* (1); *Chazarreta 7* (2); *Choussy 1595* (1); *Claussen 136* (1), *141* (3); *Coêlho de Moraes 1138* (1); *Comolli 70* (3); *Conrad & Dietrich 2066* (1), *2179* (1), *2510* (2), *2548* (2); *Contreras 170* (1); *Converse 48* (1); *Conzatti 2164* (1); *Coradin et al. 5877* (1); *Correa 72* (2); *Cortes et al. 620* (1); *Coulter 1006* (1); *Cremers 5837* (1), *7727* (1); *Croat 5804* (1), *5837* (1), *7022* (1), *15115* (1); *Crovetto 9759* (1); *Cuadros V. 1617* (1), *2008* (1), *4439* (1); *Cuatrecasas & Romero-Castaneda 25414* (1); *Cuezzo 712* (2), *860* (2), *910* (2), *2355* (2); *Curran 100* (2).

Daly et al. 6943 (1); *Davidse & Ramamoorthy 11191A* (1), *11284* (1); *Davidse et al. 29519* (1), *30528* (1); *Dawson, G. 112* (2); *Dawson, L. A. 23* (2); *Deam 53* (1); *Deam s.n.* (1); *Degen 893* (1), *1347* (1); *Degen & Zardini 597* (1); *Del Puerto 5584* (1); *Desc./Schr. 35181* (2); *Dodds 32* (1); *Dodson 5200* (1); *Dorantes L. 198* (1); *Duarte 7410* (1); *Dugand 1134* (1), *1219a* (1), *3438* (1), *6471* (1); *Duges s.n.* (1); *Dusén 7375* (1), *8677* (3), *11307* (1), *13095* (3), *Dusén s.n.* (3); *Dwyer & Liesner 12332A* (1); *Dwyer & Pippin 10092* (1).

Eastwood s.n. (1), *Eastwood s.n.* (2); *Eggers 5341* (1); *Eiten 4627* (1); *Eiten & Eiten 4627b* (1); *Ekman 1007* (1), *2462* (1), *14022* (1), *16691* (1); *Ellenberg 4437* (2); *Elvir 141* (1); *Emmons 136* (1), *213* (1); *Endres 194* (1); *Espejo & Palacios-Rios 2176* (1); *Espinosa 1894* (1); *Eyerdam & Beetle 22518* (2), *22572-p.p.* (1), *26316* (2).

Farias 97 (1); *Farney 740A* (1); *Feddema 526* (1); *Fendler 774* (1); *Fernandez 447* (1); *Fernandez & Hood 1133* (1); *Fernandez Casas & Fernandez Casas 8187* (1); *Fernandez Casas & Molero 4375* (2), *4444* (2); *Fernández N. 1582* (1); *Fiebrig, K. 492* (1), *2298* (1), *4221(4255)* (1), *4258* (1), *5703* (1), *6245* (1); *Fielding s.n.* (2); *Fisher s.n.* (1); *Flores C. 286* (1); *Fonseca et al. 731* (1); *Forero et al. 5537* (1); *Fortunato et al. 2873* (2); *Foster 840* (1), *1173* (1); *Francheschi 22537* (1); *Fries 570* (2), *1442* (1), *Fries s.n.* (2); *Fruchard 472* (2), *Fruchard s.n.* (2); *Fryxell & Lott 3248* (1); *Fuentes 2398* (2).

Galeotti 1041 (1); *Garcia, M. M. 66* (1); Garcia, *P. 676* (2), *757* (2); *García-Barriga 6366* (1); *Garwood et al. 523* (1); *Gaudichaud* ***179*** **(1)**; *Gaumer 702* (1); *Gaumer et al. 23361* (1); *Gentry, A. H. 375* (1), *486* (1), *797* (1), *830* (1), *1166* (1), *1309* (1), *2701* (1), *2783* (1), *4504* (1), *7666* (1), *7725* (1), *7855* (1), *8189* (1), *9527* (1), *21508* (3), *50935* (1), *74115* (1); *Gentry, A. H. & Aguirre 15216* (1); *Gentry, A. H. & Berry 15117* (1), *15120* (1); *Gentry, A. H. & Cuadros 47504* (1), *64753* (1); *Gentry, A. H. & Díaz 61111* (1); *Gentry, A. H. & Foster 75339* (2); *Gentry, A. H. & Hammel 71242* (1); *Gentry, A. H. & Josse 72352* (1), *72490* (1); *Gentry, A. H. & Lajones 73047* (1); *Gentry, A. H. & Núñez 69386* (1); *Gentry, A. H. & Puig-Ross 14354* (1), *14369* (1); *Gentry, A. H. & Williams-Linera 75672* (1); *Gentry, A. H. & Zardini 49197* (3), *49385* (1), *50294* (1); *Gentry, A. H. et al. 10811* (1), *25581* (1), *27090* (1), *30711* (1), *32352* (1), *40144* (1), *50193* (1), *50206* (1), *51987* (2), *59033* (1), *59265* (1), *59446* (2), *59468* (2), *71340* (1), *71456* (1), *73258* (1), *73888* (1); *Gentry, H. S. 6706* (1); *Gentry, H. S. & Gilly 10810* (1); *Gerth s.n.* (2); *Gerieski 80* (1); *Gillis 9708* (1); *Glaziou 647* (1), *4101* (2), *5946* (2), *7773* (1), *11253* (4), *12972* (4), *14113* (1), *18375* (3), *18379* (3); *Gomes 1482* (1); *Goodspeed 23229* (2); *Gragson 287* (2); *Grant 11002p.p.* (1); *Greenman & Greenman 5894* (1); *Gregg 914* (1); *Griffiths & Hawkinson 3844* (1); *Guillén & Coria 1429* (1); *Guzman 501* (1).

Haber 555 (1); *Haber & Bello C. 1620* (1), 2942 (1); *Handro s.n.* (3); *Hansen & Nee 7467* (1), *7529* (1); *Harley 22012* (1); *Harriman et al. 10841* (1); *Harris 8600* (1), *10938* (1); *Hassler 1249* (1), ***1475*** **(1)**, *1475a* (1), *1618* (1), ***1766*** **(1)**, ***2127*** **(1)**, ***2630*** **(2)**, *3569* (1), *3721* (1), *3744* (1), ***6673*** **(1)**, ***6889*** **(1)**, *7441* (1), ***8636*** **(1)**, *8956* (1), *11887* (1), *12467* (1), *Hassler s.n.* (1); *Hatschbach 1721* (1), *2614* (3), *6391* (3), *18505* (4), *25859* (1), *28475* (1), *28516* (3), *30840* (1), *37355* (1), *39175* (3), *39231* (1), *40277* (1), *40395* (3), *40634* (1), *41085* (1), *41905* (1), *42606* (1), *44445* (1); *Hatschbach & Pereira 10281* (1); *Hatschbach & Silva 48688* (1), *49108* (1), *54734* (1), *61218* (1); *Haught 2323* (1), *4169* (1); *Hawkes et al. 3199* (2); *Heithaus 121* (1); *Herald & Clark 338* (1); *Heriberto, Bro. 214* (1); *Heringer 2231* (4); *Herter 1012A* (1), *1032* (2), *1637* (2), *1893* (1); *Herzog 1094* (2), *1114* (1), *1100* (1); *Heyde & Lux 3108* (1), *4552* (1); *Hieronymus 117* (2), *914* (2), *Hieronymus s.n.* (2); *Hinton 747* (1), *8025* (1), *8506* (1), *14253* (1), *25314* (1); *Hinton et al. 7153* (1), *10185* (1), *10580* (1), *13817* (1), *13885* (1), *24182* (1); *Hioram, Bro. 1896* (1); *Hoehne 827* (3), *953* (1), *33259* (3); *Hoehne 1532* (3); *Holm-Nielsen 16022* (1); *Holst 4484* (1); *Hopkins et al. 133* (1); *Howell 10526* (1); *Hubrich 30* (2); *Huidobro 11* (2), *1297* (2), *1912* (2), *3000* (2), *3050* (2), *3114* (2), *3528* (2), *3596* (2), *3632* (2); *Humboldt & Bonpland* ***s.n.*** **(1)**; *Hunziker, A. T. 665* (2), *1004* (2); *Hunziker, A. T. & Caro 13608* (2); *Hunziker, J. H. et al. 10835* (1).

Ibarra M. & Sinaca C. 3385 (1); *Ibarrola* 3473 (2); *Iltis et al. 1135* (1); *Irigoyen 353* (2); *Irwin et al. 10343* (1), *17419* (1); *Isler 118* (2); *I. Binacional 1119* (1).

Jack 5686 (1); *Jacquemin 1931* (1); *Janzen 10239* (1), *10965* (1), *12038* (1); *Jativa 2966* (2); *Jimenez M. 3974* (1); *Jiménez & Ramírez 1472* (1); *Job 574* (2), *830* (2), *1459* (2); *Johnson 280-73* (1); *Jones & Facey 3301* (1); *Jonker-Verhoef & Jonker 569* (1); *Jörgensen 1094* (2), *1969* (2), *3452* (1); *Jurgens 5* (1); *Jurgensen 230* (1).

Keil & Lehto 6330 (2); *Kellerman 5029p.p.* (1); *Kenoyer s.n.* (1); *Kenoyer & Crum 3313* (1), *3658p.p.* (1); *Kiesling 4503* (2); *Kiesling et al. 5015* (2); *Killeen et al. 4168* (2); *King, D. O. 545* (2); *King, R. M. 445* (1), *1620* (1); *King, R. M. & Diboll 2763* (1); *King, R. M. & Soderstrom 4942* (1); *Klein 943* (1), *3232* (1), *3292* (1), *3348* (1), *3622* (1), *3741* (1), *3938* (1); *Kleinschmidt 14402* (1); *Krapovickas & Cristóbal 16514* (2); *Krapovickas & Schinini 31845* (1); *Krapovickas et al. 19944* (2), *27670* (2); *Kullberg s.n.* (2); *Kummrow 1417* (1), *2094* (1); *Kummrow & Stutts 1827* (1); *Kunkel, G. 11006* (1); *Kunkel, I. 434* (1); *Kuntze s.n.* (1), *Kuntze s.n.* (2).

Landrum 3885 (1); *Langlassé 1015* (1); *Laughlin 868* (1), *1112* (1), *1692p.p.* (1), *2025* (1); *Legname & Cuezzo 10379C* (2); *Leite 3197* (1); *León, Bro. & Roig 11440* (1); *Lespedes 5* (1); *Liebmann 8027* (1), *8028* (1); *Liesner 2546* (1), *4802* (1); *Lillo 5246* (1), *6179* (2), *18091* (1), *56973* (1); *Iglesias de Lima s.n. (RB58085)* (1); *Lindberg 153b* (1), *156a* (1); *Lindeman & de Haas 3287* (1), *5420* (1); *Löfgren 64* (1); *Lohmann 626* (1); *Lopez-Palacios 1862* (1); *Lopez-Palacios & Bautista B. 3240* (1); *Lorence et al. 3817* (1); *Lorentz 48* (2), ***120*** **(2)**, *912* (2), *1532* (2); *Lossen 28* (2); *Lott & Solis Magallanes 1247* (1); *Lott et al. 445* (1); *Lourteig 2263* (1); *Luna, F. E. 31* (2); *Luna, M. J. 11* (2); *Lund* ***786*** **(1)**, *Lund s.n.* (1); *Lundell & Lundell 7336* (1), *7554* (1); *Lyonnet 2192* (1), *Lyonnet s.n.* (1).

MacDougall H490 (1), *H501* (1), *MacDougall s.n.* (1); *Macedo 2037* (1); *Magallanes 3583* (1), *4261* (1); *Magallanes & Basurto 1854* (1); *Maldonado B. 187* (2); *Malvarez 86* (2), *255* (2), *311* (2), *1516* (2); *Manso da Silva s.n. (Martius Herbarium Florae Brasiliensis 516)* (1); *March 655* (1); *Marin*

et al. 146 (1); *Martinez 782* (1); *Martínez S. & Soto N. 1360* (1); *Martínez S. & Soto A. 18843* (1); *Martínez S. & Téllez 12824* (1); *Martino de et al. 831* (1); *Martius* ***408*** **(4)**, *Martius s.n.* (4); *Mathias 307* (1); *Mattos-Silva et al. 1509* (1); *Matuda 4405* (1), *4498* (1), *5588* (1), *27911* (1); *Maxon et al. 7828* (1); *Maxwell 266* (1); *McClintock s.n.* (2); *McCready s.n.* (1); *McFarlin 5192* (2); *McVaugh 14952* (1), *15845* (1). *15897* (1), *22580* (1); *McVaugh et al. 1501* (1); *de Mello 17A* (1), *55* (3); *Mexia 4343* (2), *5090* (1), *7245* (1); *Meyer 3848* (2). *8345* (2), *8723* (2), *10048* (2), *12753* (2), *13193* (2), *13535* (2), *Meyer s.n.* (2); *Meyer & Sleumer 15264* (2); *de Michel 64* (1), *160* (1), *226* (1); *Mikan s.n.* (1); *Miller s.n.* (1); *Molina R. 190* (1), *862* (1), *14268* (1), *14443* (1); *Monetti 12* (2), *103* (2), *1248* (2), *3098* (2); *Montalvo 3446* (1); *Moore, H. E. 5500* (1); *Moore, S. 979* (1); *Morales 2928* (1); *Morales et al. 1506* (1), *2572* (1); *Moreira 87* (1); *Morel 381* (2), *418* (2), *920* (2), *1277* (2); *Moreno 3144* (1), *4553* (1), *4895* (1), *16237* (1), *16715* (1), *18504* (1), *20014* (1), *22085* (1), *24255* (1), *24270* (1), *24279* (1); *Morillo et al. 4243* (1); *Morong 158* (1), ***749*** **(1)**; *Morrone et al. 1999* (1); *Mosén 954* (1); *Mueller s.n.* (1).

Nagel 8028 (1); *Narvaez M. & Salazar 271* (1); *Nee 30504* (1), *33550* (1), *35127* (1), *35304* (2), *35355* (1), *40596* (1), *41083* (1), *46371* (1), *47418* (1); *Nee & Taylor 28772* (1), *29641* (1); *Neill 1200* (1); *Nelson, C. et al. 7024* (1); *Nelson, E. W. 1668* (1), *2568* (1), *2615* (1), *4434* (1); *Novara 2148* (2), *8429* (2), *8502* (2); *Novara & Bruno 9525* (2); *Núñez 8123* (1), *13965* (1); *Núñez & Gentry 10272* (1); *Núñez et al. 6828* (1), *9952* (1).

O'Donell 4223 (2), *O'Donell s.n.* (2); *O'Donell & Rodríguez V. 468* (2), *877* (2); *Oersted 19* (1); *Oliveira 758* (3); *Opler 871* (1), *1767* (1); *Ortega, F. 717* (1); *Ortega, J. G. 94* (1), *7274* (1); *Ortega O., R. 666* (1); *Ortiz, B. & Martiniano 162* (1); *Ortiz, J. J. 1022* (1); *Ousset 107* (2).

Padilla 383 (1); *Palacios et al. 8800* (1); *Palmer 211* (1), *403* (1), *Palmer s.n.* (1); *Pavon s.n.* (1); *Peachmann s.n.* (2); *Pearce s.n.* (2); *Peckolt 122* (1); *Pedersen 128* (2), *2972* (2), *4273* (1), *5263* (1), *5272* (2), *7335* (2), *11527* (1), *11527A* (1), *12620* (1); *Peirano 56982* (2); *Peixoto et al. 4140* (1); *Peña-Chocarro & Arroyo 154* (4); *Pensiero & Vegetti 2721* (2); *Peredo 361* (1); *Pereira & Hatschbach 7664* (1); *Petersen & Hjerting s.n.* (2); *Pfeifer 1686* (1); *Pflanz 2001* (1); *Pickel 3586* (1); *Pieratti 11537* (2), *4169* (2), *11537* (2), *Pieratti s.n.* (2); *Pittier 340* (1), *8906* (1), *13956* (1); *Pizzaia et al. 27* (1); *Plowman 12734* (1); *Plowman & Ramírez R. 11263* (1); *Plowman & Vaughan 5358* (1); *Pollard s.n.* (1); *Powell 268* (1); *Prance 23162* (1); *Prance et al. 26223* (1); *Prevost 1458* (1); *Pringle 3060* (1), *3074* (1), *8273* (1); *Proctor 26476* (1), *31733* (1); *Purpus 7289* (1), *8772* (1).

Quevedo S. 19 (1).

Raben 161 (1), *504* (1), *Raben s.n.* (1); *Rambo 29428* (1), *44852* (1), *44956* (1), *57534* (1); *Ramirez 705* (1), *878* (1); *Reales 173* (2); *Regnell 336* (1), *2287* (1), *I-287* (1); *Reiche 704* (1); *Reineck & Czermak 136* (1); *Reitz 2721* (1), *5284* (1), *6545* (1); *Reitz & Klein 936* (1), *1112* (3), *4085* (1), *7286* (3), *7609* (1), *8054* (1), *11760* (1), *14055* (1), *14502* (1), *14751* (1), *14951* (1); *Renvoize et al. 3262* (1), *3381* (2); *Reyes-García & Hampshire 1916* (1); *Reyes-García & Martínez 27* (1), *30* (1), *41* (1), *128* (1); *Reyes-García et al. 1560* (1); *Richardson 1346* (1); *Richter s.n.* (HB-*29882*) (1); *Riedel s.n.* (1); *Riggs 122* (2); *Robert 749* (1); *Robertson & Austin 7* (1); *Robleto 1213* (1); *Rodrigo 252* (2), *2063* (2); *Rodriguez 47* (1), *1163* (1); *Rojas (Hassler no. ser.)* ***10708*** **(1)**, ***10708a*** **(1)**; *Romero-Castañeda 9261* (1); *Romo 471* (1); *Rondeau 259* (1); *Rose 1696* (1), *1818* (1); *Rose & Rose 22612* (1); *Rose et al. 8543* (1), *14437* (1), *Rose s.n.* (1); *Rovirosa 552* (1); *Rowlee & Rowlee 280* (1); *von Rozynski 442* (1), *630* (1); *Rugel 315* (1), *861* (1); *Runyon 5435* (1), *5439* (1); *Rusby 104* (1); *Rzedowski 21883* (1).

Sacco da Costa 446 (1); *Saenz & Ryhr 499* (2); *Saer 236* (1); *Sagot 405* (1); *Saint-Yves 166* (2); *Saldías et al. 1324* (1); *Sanders et al. 4967* (1); *Sandino 3108* (1), *3138* (1), *4403* (1); *Santos et al. 84* (1); *dos Santos* ***574*** **(4)**; *Scala s.n.* (2); *Schinini 5024* (1), *7524* (2), *11632* (2); *Schinini & Mroginski 4464* (2), *4736* (2); *Schinini et al. 23837* (1); *Schott s.n.* (2); *Schreiter 281* (2), *3590* (1), *8924* (2), *10891* (1), *10892* (1), *56979* (2), *56981* (2); *Schrolthy 62* (1); *Schulz, A. G. 1617* (2). *1637* (2), *7016* (1), *7340* (2), *8531* (1), *8936* (2), *9636* (1), *10587* (2); *Schulz, C. L. 839* (2); *Schunke V. 3750* (1), *5744* (1), *5859* (1); *Schwarz 72* (2), *150* (2), *1406* (1), *1414* (1); *Schwarzer s.n.* (1); *Scolnik 48* (2); *Sehnem 3489* (1); *Seibert 1149* (1), *1150* (1), *1430* (1), *1448* (1); *Seidel 598* (1); *Seigler et al. 13589* (1); *Seler & Seler 3170* (1); *Sellow* ***1618 (736)*** **(1)**, ***3271*** **(1)**, *Sellow* ***s.n.*** [Herbarium number ***332***] **(3)**, *Sellow s.n.* (1); *Servin 1347* (1); *Sessé et al. 2404* (1); *Shafer 1396* (1), *12082* (1), *12171* (1); *Sierra 93* (1); *Silva, A. F. et al. 8857* (1); *Silva, J. M. & Hatschbach 24* (3); *Silva, J. M. & Ribas 408* (1); *Silva, J. M. & Ruas 80* (3); *Silva 1674* (1); *Silverstone S. 2570* (1), *3249* (1); *Sinaca C. 1509* (1); *Sinaca C. & Chigo S. 719* (1); *Sinaca C. & Sinaca D. 1557* (1); *Smith, A. 2854* (1); *Smith, C. L. 1961* (1); *Smith, D. N. & García 13874* (1); *Smith, D. N. et al. 14211A* (1); *Smith, H. H. 350* (1); *Smith, J. D. 2690* (1), *6682B* (1); *Smith, L. B. & Klein 8178* (1), *8598* (1), *10697* (1), *11033* (1), *13258* (1), *13879* (1); *Solheim 1690* (1); *Solomon 2426* (1), *12422* (1), *14713* (1); *Solomon & Nee 12607* (1); *Solomon & Solomon 4040* (2); *Solomon et al. 6808* (1); *Soria & Mereles 2745* (1); *Soria y Vera 2846* (1); *de la Sota 238* (2), *437* (2), *3445* (2); *Soto Nunez 981* (1); *Soto Nunez & Boom 2129* (1); *Soukup 898* (1), *5659* (2), *5661* (1); *Soule & Brunner 2358* (1); *Sparre 777* (2); *Spellman et al. 553* (1); *Spichiger & Loizeau 1525* (2); *Spichiger et al. 2070* (2), *2075* (2); *Splitgerber* ***544*** **(1)**; *Spruce 4539* (1); *Standley 11639* (1), *20883* (1), *21029* (1), *21692* (1), *22153* (1), *24212* (1), *26055* (1), *76094* (1); *Stanton s.n.* (1); *Stearn 792* (1); *Steere 1018* (1); *Steinbach 1729* (1), *3061* (1), *3218* (1), *5254* (1), *7428* (1); *Stergios 8629* (1); *Stevens 5705* (1); *Stevens & Montiel 17367* (1); *Steyermark 94348* (1), *Steyermark s.n.* (1); *Steyermark et al. 122887* (1), *123298* (1), *123465* (1); *Stork et al. 10466* (1); *Stuckert 14694* (2), *19767* (2); *Sucre et al. 10598* (1); *Sullivan 689* (1).

Tate 565 (1); *Teague 314* (1), *649A* (1); *Tenorio L. et al. 368* (1); *Tessmann 4576* (1); *Thieme 5394* (1); *Thomas s.n.* (1); *Timana & Jaramillo 2688* (1), *2694* (1); *Timmermann 71123* (2); *Ton 3164* (1), *3757* (1); *Tonduz 8906* (1), *13975* (1); *Torres, M. A. 126* (1); *Torres C., R. & Campos V. 13915* (1); *Torres C., R. & Cedillo T. 2800* (1); *Torres C., R. et al. 532* (1); *Townsend 927* (1); *Tressens et al. 4680* (1); *Triana 4124-5* (1); *Trujillo et al. 16561* (1); *Tucker 591* (1); *Tún O. 2608* (1); *Turner 2053* (1); *Tweedie 493* (2), *Tweedie s.n.* (2).

Ucan 4166 (1); *Ule 6812* (1).

Valerio-Rodriguez 814 (1); *Valverde 555* (1); *van der Werff et al. 8618* (1); *Vanni & Cáceres 629* (1), *699* (1); *Varela 12* (2), *744* (2); *Vavrek & Perez 233* (1); *Vazquez, F. 108* (1); *Vázquez T., M. et al. 3995* (1); *Velazquez L. 278* (1); *Ventura, E. & López 9256* (1); *Ventura A., F. 2698* (1), *3725* (1), *3832* (1), *8349* (1), *11651* (1), *16189* (1); *Venturi 227* (2), *5161* (2), *5395* (2), *5395* (2), *5626* (1), *7212* (2), *7508* (2), *7893* (2), *9665* (2), *9665* (2), *10342* (2); *Vianna & Leal 1589* (1); *Vianna et al. 1582* (1); *Villafañe 35* (2), *126* (2), *745* (2); *Vincent 5454* (1); *Vogel, C. 802* (1); *Vogl, P. & Sussenguth 384* (1); *Voorhies & Sanchez 2-5* (1).

Wachenheim 73 (1); *Walker 1223* (1); *Wall 9* (2); *Walter & Walter 302* (2); *Walther s.n.* (1); *Webster et al. 11667* (1), *25892* (1); *Weir 70* (1); *Wendt et al. 4015* (1); *West 6132* (2),

8364 (1); *Widgren 241* (1), *241* (1), *639* (1), *Widgren s.n.* (1); *Wight 1693* (2); *Wilbur & Wilbur 2091* (1); *Williams, J. H. 42* (1); *Williams, L. 9804* (1); *Williams, L. O. & Molina R. 12704* (1), *16708* (1); *Wilson 1113* (1); *Wright, C. 1337* (1), *3051* (1), *3057* (1); *Wright, D. s.n.* (1); *Wright, W. s.n.* (1); *Wullschlaegel 338* (1), *Wullschlaegel s.n.* (1).

Yakitaku 1 (3); *Yanagizawa s.n. (SP8986)* (1); *Young & Eisenberg 269* (1), *434* (1), *463* (1).

Zamora 2257 (1); *Zardini 1594* (2), *5062* (1), *5089* (1), *6825* (1), *13398* (1), *20065* (1); *Zardini & C. Velásquez 11716* (1), *12532* (1), *14701* (1), *19278* (1), *19799* (1), *20297* (1), *20467* (1), *22197* (1); *Zardini & E. Velázquez 19175* (1), *21342* (1); *Zardini & H. Velázquez 22636* (1); *Zardini & R. Velásquez 11507* (1), *17191* (1); *Zardini & U. Velázquez 15544* (1); *Zardini et al. 9073* (1); *Zarucchi & Cuadros 4112* (1); *Zomer 266* (1); *Zuloaga et al. 2061* (1).

CHROMOSOME NUMBERS AND KARYOTYPES IN ASTERACEAE[1]

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Abstract

Chromosome numbers were determined from microsporocytes (meiotic counts) and root-tip cells (mitotic counts) in 201 collections of 51 genera and 119 specific and infraspecific taxa belonging to nine tribes of Asteraceae and *Acicarpha spathulata* R. Br. of Calyceraceae from Australia, Bolivia, Brazil, Chile, Mexico, and the United States. These include the first reports and new numbers for the basal members of the Barnadesieae and Mutisieae. First reports are provided for 45 taxa, including five genera, *Dasyphyllum* HBK ($2n = 54$), *Dithyrostegia* A. Gray ($2n = 14$), *Epitriche* Turcz. ($2n = 10$), *Revealia* R. M. King & H. Rob. ($2n = 22$), and *Stifftia* J. C. Mikan ($2n = 51$). In addition, new chromosome numbers are established at the generic level in the genera *Barnadesia* Mutis ($2n = 54$), *Eremanthus* Less. ($2n = 34, 30$), *Macvaughiella* R. M. King & H. Rob. ($2n = 24$), and *Trichocline* Cass. ($2n = 38$), and in 12 additional species. Remaining counts augment and agree with previously reported determinations. The base chromosome numbers of Calyceraceae and Asteraceae are discussed in published records and our original counts.

Key words: Asteraceae, Brazil, chromosome number, cytotaxonomy, genome size, karyotype, phylogeny.

Chromosomal information has contributed extensively to our understanding of relationships within the diverse Asteraceae and has resulted in heightened awareness of groups in need of taxonomic re-evaluation. Most previous studies on the chromosomes of this family were conducted on plants in temperate regions of the Northern Hemisphere, although there is more species diversity in Central America and South America.

Recent molecular studies have suggested that Barnadesieae is the most primitive tribe in the Asteraceae, followed by Mutisieae, while Calyceraceae is sister to Asteraceae (Jansen & Palmer, 1987, 1988; Kim & Jansen, 1995; Bayer & Starr, 1998; Kim et al., 2002; Panero & Funk, 2002). Most members of Barnadesieae and Mutisieae are concentrated in South America and are relatively poorly known cytologically. In this paper, we report original chromosome counts from 201 accessions representing 120 species and varieties, including first reports for 45 taxa of Asteraceae and one species of Calyceraceae.

Materials and Methods

A total of 201 accessions attributed to 51 genera and 119 specific and infraspecific taxa of nine tribes of Asteraceae and *Acicarpha spathulata* R. Br. of Calyceraceae were collected from Australia, Bolivia, Brazil, Chile, Mexico, and the United States. Chromosome counts were obtained from either floral buds fixed in a 3:1 ethanol-acetic acid fixative in the field or root tips obtained from seedlings grown from cypselae of known provenance in the greenhouse of Kobe University. Excised root tips about 1 cm in length were pre-treated with cold water at 0°C for 24 hours and fixed in 3:1 ethanol-acetic acid for 1–5 hours at 5°C. Haploid karyotypic idiograms are based on the means of 10 measurements for each chromosome.

Although Turner (1987) and Bremer (1994) have treated *Revealia* R. M. King & H. Rob. and *Cronquistia* R. M. King as synonyms of *Carphochaete* A. Gray, in the present study, the two are treated as separate genera with different chromosome numbers

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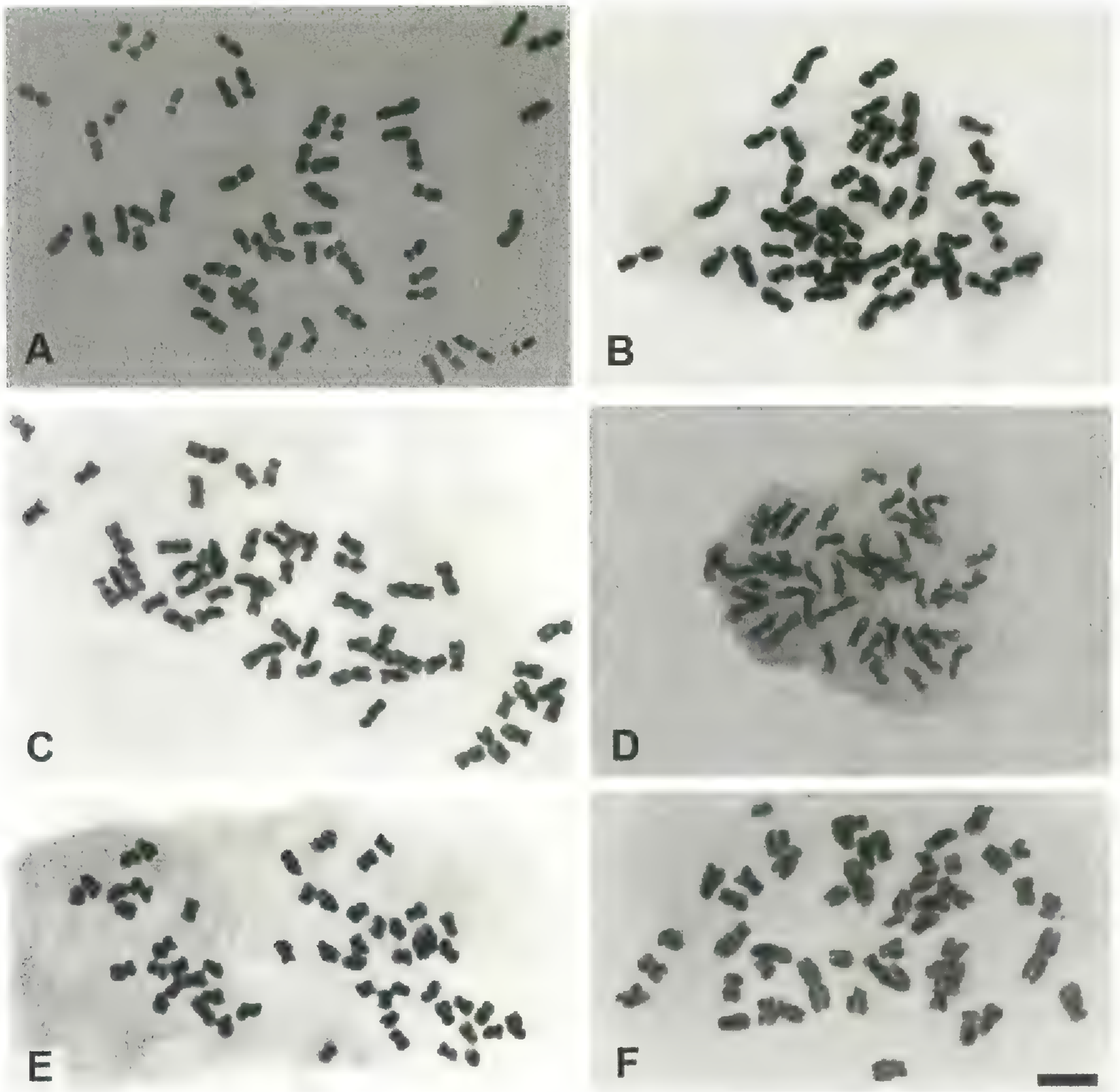

Figure 1. Photomicrographs of somatic metaphase chromosomes. Scale bar = 5 μm. —A. *Dasyphyllum spinescens* ($2n$ = 54). —B. *Dasyphyllum brasiliense* ($2n$ = 54). —C. *Barnadesia caryophylla* ($2n$ = 54). —D. *Stifftia chrysantha* ($2n$ = 54). —E. *Trixis praetans* ($2n$ = 54). —F. *Mutisia campanulata* ($2n$ = 52).

and habits. Voucher specimens have been deposited in the Centro de Pesquisas de Historia Natural Herbario Goro Hashimoto (GHSP, Brazilian specimens), KANA (Bolivian and Chilean specimens), MEL (Australian specimens), MEXU (Mexican specimens), KYO (Brazilian and Mexican specimens), and TI (Australian specimens). Indices consulted for previous chromosome number determinations include: Fedorov (1974), Moore (1973), Goldblatt (1981, 1984, 1985, 1988), Goldblatt and Johnson (1990, 1991, 1994, 1996, 1998, 2000, 2003), and <http://www-asteraceae.cla.kobe-u.ac.jp>. We have cited only these in order to save space and still provide a means of access to the primary literature.

Results and Discussion

Chromosome numbers of 201 collections representing 51 genera and 119 specific and infraspecific taxa belonging to nine tribes of Asteraceae and *Acicarpha spathulata* of Calyceraceae were determined. Among them, the chromosome numbers of 45 species and/or infraspecific taxa and five genera (*Dasyphyllum* Kunth [$2n$ = 54], *Dithyrostegia* A. Gray [$2n$ = 14], *Epitriche* Turcz. [$2n$ = 10], *Revealia* [$2n$ = 22], and *Stifftia* J. C. Mikan [$2n$ = 54]) have not been previously counted. Chromosome numbers for *Barnadesia* Mutis ex L. f. ($2n$ = 54), *Eremanthus* Less. ($2n$ = 34, 30), *Macvaughiella* R. M. King & H. Rob. ($2n$ = 24), *Trichocline* Cass. ($2n$ = 38), and 14 additional species differ from those previously reported; new base chromosome numbers are established for those species. Among taxa reported here, three show infraspecific polyploidy (Appendix 1). Photomicrographs of the mitotic chromosomes of six taxa are displayed in Figure 1, and the idiograms of the mitotic chromosome of 17 taxa are displayed in Figure 2. The characteristics of karyotypes of 17 taxa are presented in Table 1.

The tribal arrangements of Asteraceae listed in Appendix 1 and Table 1 follow those of Panero and Funk (2002).

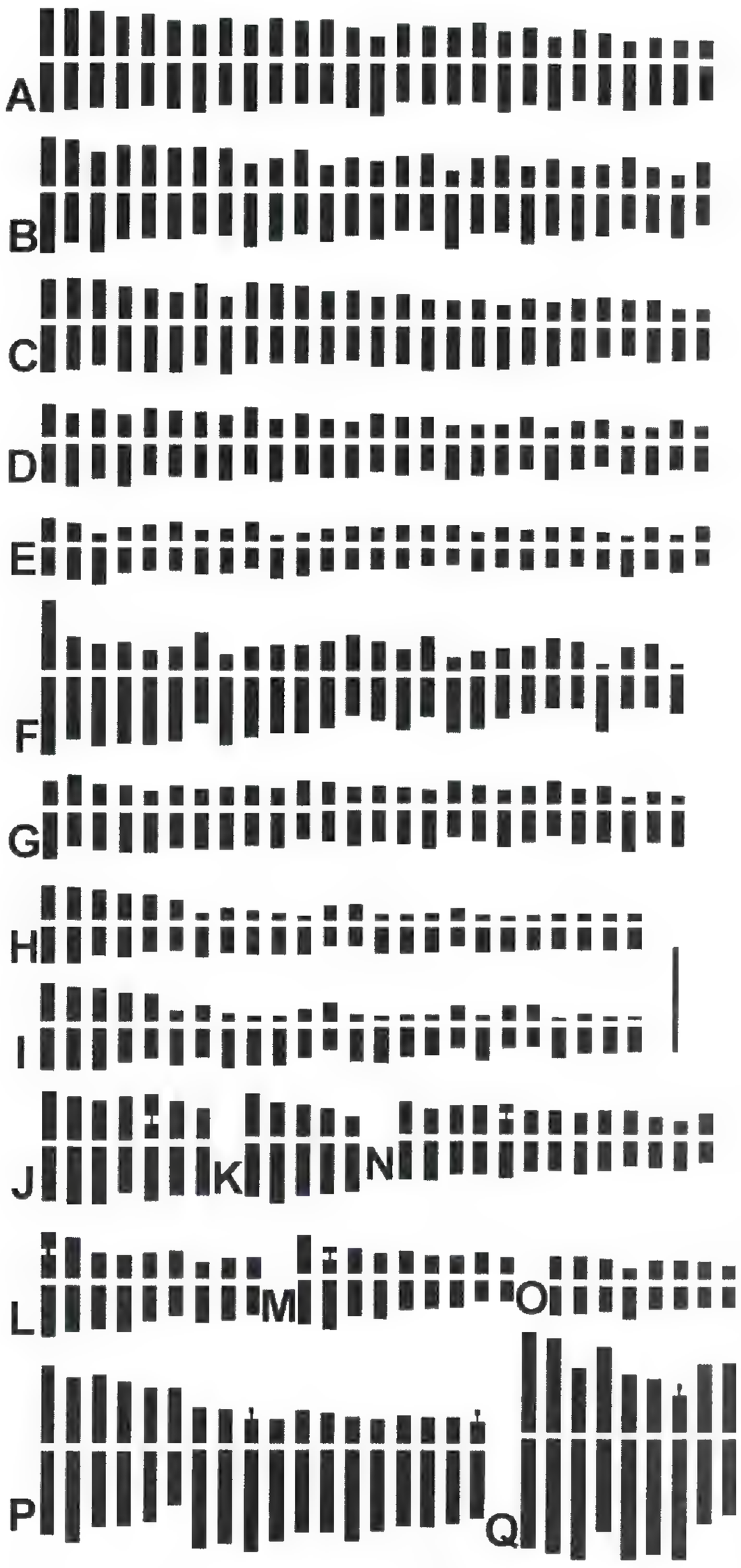

Figure 2. Haploid karyotypic idiograms. Scale bar = 5 µm. —A. *Dasyphyllum spinescens* (n = 27). —B. *Dasyphyllum brasiliense* (n = 27). —C. *Barnadesia caryophylla* (n = 27). —D. *Stifftia chrysantha* (n = 27). —E. *Trixis praetans* (n = 27). —F. *Mutisia campanulata* (n = 26). —G. *Mutisia* sp. (n = 26). —H. *Chaptalia integrifolia* (n = 24). —I. *Chaptalia nutans* (n = 24). —J. *Symphyotrichum graminifolium* (n = 7). —K. *Epitriche demissus* (n = 5). —L. *Aster squamata* (n = 9). —M. *Notiastrum acuminatum* (n = 9). —N. *Dyssodia papposa* (n = 13). —O. *Thymophylla aurea* (n = 8). —P. *Marshalia mohrii* (n = 18, diploid karyotypic idiogram). —Q. *Espejoa mexicana* (n = 9).

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Acicarpha spathulata has a chromosome number of $2n$ = 8II, which is in accord with numbers previously reported.

ASTERACEAE

Barnadesieae. First reports are provided for seven specific and infraspecific taxa of *Dasyphyllum* and *Barnadesia caryophylla* (Vell.) S. F. Blake. In *Barnadesia*, chromosome numbers have been reported for eight species. They vary from n = 12 (Olsen, 1980) to $2n$ = 62 (Cristobal, 1986). In *B. caryophylla*, we found a chromosome number of $2n$ = 54. This is the first report of this number for this genus, and it matches or closely approximates previously reported numbers for other genera such as *Arnaldoa* Cabrera (n = 24–27 in Stuessy & Sagastegui, 1993) and *Chuquiraga* Juss. (n = 27 and/or $2n$ = 54 in Diers, 1961; Wulff, 1990). In addition, $2n$ = 54 is reported here for the first time for seven *Dasyphyllum* taxa. Reports for taxa of *Dasyphyllum* and *Barnadesia* establish a new base chromosome number of x = 27 for both genera here.

Mutisieae. First reports are provided for *Chaptalia graminifolia* (Dusen) Cabrera ($2n$ = 48 + 0–7Bs), *C. runcinata* Kunth ($2n$ = 48), *Jungia sellowii* Less. (n = 20II), *Mutisia campanulata* Less. ($2n$ = 52), two varieties of *Stifftia chrysantha* Mikan ($2n$ = 54), *Trichocline catharinensis* Cabrera ($2n$ = 38), and *Trixis praestans* (Vell.) Cabrera ($2n$ = 54). All of the genera examined have high chromosome numbers. Counts of $2n$ = 54 for the genera *Stifftia*, *Trixis* P. Browne, *Acourtia* D. Don (Vuilleumier, 1969; Pinkava & Keil, 1977; Powell & Powell, 1978; Ward, 1983; Carr et al., 1999; Strother & Panero, 2001), *Plazia* Ruiz & Pav. (Covas & Schnack, 1946), and *Proustia* Lag. (Covas & Schnack, 1946) agree with those of four genera of Barnadesieae. Our counts of $2n$ = 54, as well as many previous reports of n = 27 for *Trixis* species, differ from the report from Carr et al. (1999) for *T. inula* Crantz ($2n$ = 28II). Chilean and Brazilian *Mutisia* L. f. species have chromosome numbers of $2n$ = 52, which agree with those of *M. acuminata* Ruiz & Pav. (Waisman et al., 1984; Carr et al., 1999), *M. clematis* L. f. (Powell & King, 1969), *M. coccinea* A. St.-Hil. (Gibbs & Ingram, 1982), *M. ledifolia* Decne. ex Wedd. (Waisman et al., 1984), and *M. orbignyana* Wedd. (Fernández Casas, 1981) and are different from previous counts for other species (Diers, 1961; Turner et al., 1979). Grau (1987) reported $2n$ = 46 for five Chilean *Mutisia* species. Thus, our count of $2n$ = 52 for Chilean *Mutisia* species is a new report. Previous chromosome counts for *Trichocline* species were reported to be n = 18 (Covas & Schnack, 1947; Zardini, 1975) and n = 20 (Turner et al., 1979). Our count for *Trichocline catharinensis* establishes a new base number, x = 19, at the generic level. Kim et al. (2002) showed that Mutisieae is polyphyletic based on

Table 1. Taxa, chromosome number, total haploid karyotypic length, range of chromosome size, ratio of chromosome size, mean arm ratio, and life form. TKL, total haploid karyotypic length; range, the longest chromosome to the shortest chromosome; ratio, the longest chromosome to the shortest chromosome; SE, standard error; SD, standard deviation; MAR, mean arm ratio (total long arm length to total short arm length); LF, life form; T, tree; S, shrub; L, liana; P, perennial; A, annual.

Taxon	$2n$	TKL (μm)			Range (μm)	Ratio	MAR	LF
		Total	SE	SD				
Dasyphyllum spinescens	54	185.46	10.04	22.45	4.44–2.48	1.79	1.46	T
Dasyphyllum brasiliense	54	185.32	9.29	20.78	5.03–2.44	2.06	1.49	T
Barnadesia caryophylla	54	161.40	5.86	13.11	4.02–1.92	2.10	1.53	S
Stifftia chrysantha	54	126.52	2.96	6.62	3.10–1.72	1.80	1.55	S
Trixis praestans	54	92.58	2.64	5.91	2.20–1.34	1.64	1.71	S
Mutisia campanulata	52	176.66	3.88	8.68	6.78–1.88	3.61	2.17	L
Mutisia sp.	52	116.72	1.96	4.38	3.09–1.69	1.83	2.32	L
Chaptalia integrifolia	48	82.28	6.10	13.63	3.08–1.20	2.57	2.21	P
Chaptalia nutans	48	95.68	4.76	10.65	3.71–1.19	3.12	2.31	P
Dithyrostegia amplexicaulis	14	58.52	0.67	1.50	4.61–3.59	1.28	1.39	A
Epitriche demissus	10	37.84	0.80	1.78	4.34–3.07	1.41	1.77	A
Aster squamatus	18	50.38	2.58	5.77	3.89–2.09	1.86	1.42	P
Voticastrum acuminatum	18	41.86	1.30	2.91	3.58–1.55	2.31	1.43	P
Marshallia mohrii	36	202.64	6.68	14.93	7.50–4.10	1.83	2.13	P
Espejoa mexicana	18	147.16	4.98	9.13	9.66–6.72	1.44	1.61	A
Dyssodia papposa	26	38.98	1.06	2.38	3.17–1.79	1.77	1.32	A
Thymophylla aurea	16	28.70	0.64	1.44	2.08–1.53	1.36	1.11	A

the *ndhF* sequence variation. Panero and Funk (2002) supported the polyphyly of the old Mutisieae and proposed to allocate members of Cardueae and Mutisieae into the following group and subfamilies: "The *Stifftia* group," Mutisioideae, Gochnatioideae, Hecastocleioideae, Carduoideae, Pertyoideae, and Gymnarrhenoideae, based on the comparison of 13380 bp of cpDNA. "The *Stifftia* group" follows the basal Barnadesioideae, a relationship consistent with the same chromosome numbers. The base chromosome number for Mutisieae, including "the *Stifftia* group," may be $x = 27$. The chromosome numbers $2n = 52$, $2n = 48$, and $2n = 38$ may have been derived through dysploidal reduction.

Vernonieae. First reports are provided for *Eremanthus bicolor* (DC.) Baker ($2n = 30$) and three species of *Vernonia* Schreb. There are three unidentified specimens of *Eremanthus*; *Eremanthus* species 1 has a chromosome number of $2n = 30$, but species 2 and species 3 have chromosome numbers of $2n = 34$ and/or $2n = 17$II. Turner et al. (1979) reported $n = 15$ for Brazilian *E. elaeagnus* Sch. Bip. Jones (1982) reported $2n = 36$ for Brazilian *E. reflexo-auriculatus* G. M. Barroso. Thus, three base chromosome numbers ($x = 15$, 17, and 18) have been reported for *Eremanthus*. We report $2n = 17$II or $2n = 34$ for six species and one unidentified specimen of *Vernonia*. The chromosome base numbers of New World *Vernonia* have been reported to be $x = 17$, 16, 15, 14, and 10 (Ruas et al., 1991; Dematteis, 1997). Dematteis (1998) and Dematteis and Fernandez (2000) reported $2n = 32$ for all species of *Vernonia* they examined.

Gnaphalieae. The additional descending dysploidy of $x = 13$, 12, and 6 in *Angianthus* J. C. Wendl., of $x = 14$, 12, and 11 in *Erymophyllum* Paul G. Wilson, and of $x = 14$ and 13 in *Podotheca* Cass. was found in a previous study (Watanabe et al., 1999a). The Australian members of Gnaphalieae we examined have lower chromosome numbers ($n = 13$, 12, 7, and 5), which are lower than those present on other continents ($n = 14$; Watanabe et al., 1999a).

Astereae. First reports are provided for *Baccharis pentodonta* Malme and *B. triplinervis* Baker. All species of shrubby *Baccharis* L. examined are diploids based on $x = 9$. All of the previous counts for *Symphyotrichum graminifolium* (Spreng.) G. L. Nesom reported as *Aster squamatus* (Spreng.) Hieron. have

been $n = 10$ or $2n = 20$ (Schnack & Covas, 1947; Kamel, 1996), except those by Coleman (1968, $n = 9$–10) and Spooner et al. (1995, $n = 5$). The present study found chromosome counts of $2n = 18 + 0$–2Bs. The longest chromosome pair has a large trabant at the end of a short arm (Fig. 2L). Previous studies may have counted this trabant or B chromosome as an independent chromosome.

Plucheeae. *Pluchea sagittalis* (Lam.) Cabrera has $n = 10$II. As noted by Watanabe et al. (1999a), all members reported for this tribe, excluding some erroneous counts, have chromosome counts of $2n = 20$ or polyploid numbers based on $x = 10$.

Helenieae. Two previous counts for *Thymophylla pentachaeta* (DC.) Small var. *hartwegii* (A. Gray) Strother (as *Dyssodia hartwegii* (A. Gray) B. L. Rob.) were $n = 26$ (Johnston & Turner, 1962; De Jong & Longpre, 1963). This is the first chromosome number of $2n = 26$ for this variety, although Strother (1989) has stated that all *Thymophylla* Lag. species have chromosome numbers based on $x = 8$. Strother (1989) reported $2n = 16$II and $2n = 32$ for Texas *T. pentachaeta*. Our two Mexican accessions of this variety had counts of $2n = 24$. The monotypic *Espejoa mexicana* DC. has a chromosome number of $2n = 18$. *Marshallia mohrii* Beadle & F. E. Boynton has a chromosome number of $2n = 36$, which is a tetraploid based on $x = 9$.

Heliantheae. The first report, $2n = 17$II, is provided for *Verbesina clausseni* Sch. Bip. ex Baker and agrees with previous reports for other species of the genus.

Eupatorieae. First reports are provided for species of *Ageratina* Spach (1), *Carphochaete* (1), *Chromolaena* DC. (4), *Decachaeta* DC. (1), *Praxelis* Cass. (1), *Revealia* (1), and *Stevia* Cav. (9). Some species of *Chromolaena* are polyploids and apomicts; *C. kleinii* (Cabrera) R. M. King & H. Rob. is a diploid based on $x = 10$ and is probably sexual. For the first time, hexaploidy ($2n = 60$) is documented for *Chromolaena laevigata* (Lam.) R. M. King & H. Rob. Sundberg et al. (1986) reported $n = 16$ for *D. pyramidalis* (B. L. Rob.) S. D. Sundb., C. P. Cowan & B. L. Turner (= *Erythradenia pyramidalis* (B. L. Rob.) R. M. King & H. Rob). It is confirmed to have a chromosome number of $2n = 32$. A previous chromosome count for *Gyptis crassipes* (Hieron.) R. M. King & H. Rob. was reported as $n = 30$ (as *Eupatorium lanigerum* Hook. & Arn.) by Coleman (1968). Diploidy ($2n = 30$) is documented for the first time in this species. Our count of $2n = 24 + 4$–8Bs for *Macvaughiella mexicana* (Sch. Bip.) R. M. King & H. Rob. is different from a previous count of $n =$ ca. 13 (King et al., 1976). Our accession of *Microspermum nummularifolium* Lag. is a triploid with a chromosome number of $2n = 36$; triploidy is documented for the first time in this species. *Praxelis missiona* (Malme) R. M. King & H. Rob., with a chromosome number of $2n = 80$, is an octoploid based on $x = 10$. The diploid chromosome number of $n = 10$ has been reported only for Bolivian *P. clematidea* (Griseb.) R. M. King & H. Rob. by Wulff et al. (1996).

Stevia includes four basic life forms: woody shrubs, plants with woody caudices and herbaceous stems, rhizomatous perennials, and annuals, all of which have been found in Mexico. All Mexican species of shrubs or shrublets are sexual diploids with chromosome numbers of $2n = 24$, while all Mexican species of annual herbs are also sexual diploids, but their chromosome numbers vary from $2n = 24$ to $2n = 8$. North and Central American perennial *Stevia* are diploids or agamospermous polyploids based on $x = 11$ or 12 (Watanabe et al., 2001). The chromosome number of $n = 17$ reported by Grashoff et al. (1972) for the Mexican perennial *S. purpusii* B. L. Rob. is probably a miscount. It is difficult to determine the chromosome number from the irregular meiotic chromosome configurations of odd polyploids. Our accession of Mexican *S. purpusii* has a chromosome number of $2n = 36$ and is a triploid based on $x = 12$. Native South American species of *Stevia* are perennials, and mostly diploids based on $x = 11$. The chromosome number of $n = 12$ for *S. organensis* Gardner reported by Coleman (1970) is possibly erroneous. Our accession collected from the same site as that of Coleman (1970) had a chromosome number of $2n = 22$. Colombian *S. lucida* Lag., with a chromosome number of $n = 12$, is a shrub and is basically a Central American species. Three polyploids reported from South America are also basically Central American (*S. elatior* HBK by Jansen et al., 1984; *S. ovata* Willd. by Turner et al., 1967; *S. serrata* Cav. by Powell & King, 1969). The occurrence of polyploidy in Peruvian *S. andina* B. L. Rob. ($n =$ ca. 22, Turner et al., 1967) and Bolivian *S. bangii* Rusby ($2n = 44 + 0$–5Bs, present study) may represent sporadic origins (a single count, respectively) because their distribution is restricted to small areas instead of the widespread prevalence of agamospermous polyploids in North and Central American.

KARYOTYPES

Figure 2 contains haploid (except for *Marshallia* Schreb.) karyotypic idiograms. In Table 1, we report the mean total haploid karyotypic length with standard errors, standard deviations, ranges of longest and shortest chromosome, arm ratios for each karyotype, mean arm ratio (total long arm length to

total short arm length), and life form. The total haploid karyotypic length of *Dasyphyllum* and *Barnadesia* has been shown to be significantly longer than those of members of Astereae and Anthemideae, with $n = 9$ reported in the literature (Table 1; Watanabe et al., 1999b). The karyotypes of members of *Dasyphyllum*, *Barnadesia*, *Stifftia*, *Trixis*, and *Mutisia* do not have the duplicated homologous chromosome sets that would suggest autopolyploid origin. Their karyotypes are well diverged even if they have polyploid origins. The base number for Barnadesieae and Mutisieae may be $x = 27$, but the possibility that they may be hexaploids based on $x = 9$ cannot be discounted, as we mention later.

From our results (Figs. 1–2; Appendix 1; Table 1), it is apparent that there is a great diversity of karyotypes. For example, the karyotypes of *Barnadesia* and *Dasyphyllum* are unimodal and contrast with the bimodal karyotypes of *Mutisia campanulata* and *Trichocline catharinensis*, which have one pair of large chromosomes and 25 or 18 pairs of small chromosomes, respectively. *Mutisia campanulata* has three pairs of nearly telocentric chromosomes, and *Chaptalia* Vent. species *C. integrifolia* (Cass.) Baker and *C. mandonii* (Sch. Bip.) Burkart have 13 to seven pairs of nearly telocentric chromosomes, respectively. Lower chromosome numbers and asymmetric karyotypes of Mutisieae may have been derived through dysploidal reduction from a base of $x = 27$ resulting from structural rearrangements. *Marshallia* is basal in the Helenieae (Baldwin et al., 2002) and has a base chromosome number of $x = 9$. It has an extraordinarily large genome size as shown in Figure 2P and Table 1. (This accession is an allotetraploid karyotypically, and its idiogram and total karyotypic length are given as "diploid.") The smaller chromosome number and genome size (= total haploid karyotypic length) may have been selected in annuals such as *Thymophylla aurea* (A. Gray) Greene, as well as those in many other genera of Asteraceae (Watanabe et al., 1999a, b). The monotypic *Espejoa* DC. is an annual and also has a large genome size (Fig. 2Q and Table 1). It is desirable to estimate the isozyme number or the state of ploidal level for both *Marshallia* and *Espejoa* to determine whether they are "true" diploid states or not.

THE ANCESTRAL BASE CHROMOSOME NUMBER OF ASTERACEAE AND SOME RELATED LIFE FORMS

Among papers that have dealt with the ancestral base chromosome number of Asteraceae, most have suggested $x = 9$, largely because this is the modal number based on more than 30% of all counted species (Solbrig, 1977). Support for $x = 9$ comes from reports for taxa of Astereae and Anthemideae collected from temperate regions of the Northern Hemisphere, where researchers have sampled most intensively in the past. The sister family Calyceraceae has base chromosome numbers of $x = 13$, 15, 18, and 21 in three of four genera examined (Covas & Schnack, 1947; Rahn, 1960; Turner, 1978; Moore, 1981), in addition to $n = 8$, which was reported for two species of *Acicarpha* Juss. (Sugiura, 1936, 1937; Raven et al., 1965; Rodriguez Correira et al., 1977; Benko-Iseppon, 1992; present study). De Vore (1994; cited by Stuessy et al., 1996) treated *Acicarpha* as the most primitive genus within Calyceraceae. But *A. spathulata* ($n = 8$), one of the five species of the genus, is a specialized succulent plant growing on the coastal sands. In Barnadesieae, the chromosome numbers have been reported for six of nine genera. Among them, the perennial monotypic genus *Schlechtendahlia* Benth. & Hook. f. has a chromosome number of $n = 8$ (Cialdella & Lopez de Kresling, 1981; Hunziker et al., 1989), and the five remaining genera have chromosome numbers of $n = 24$, 25 (*Doniophyton* Wedd., annual; Wulff, 1990), and $n = 27$ for *Arnaldoa*, *Barnadesia*, *Chuquiraga*, and *Dasyphyllum*. Based on cladistic analysis of morphological characters, Bremer (1994) and Stuessy et al. (1996) noted that *Schlechtendahlia* and *Dasyphyllum* were primitive and basal in subfamily Barnadesioideae. Stuessy et al. (1996) treated the members of both sections of the subgenus *Dasyphyllum* as having the life form of arching shrubs. Among the accessions we examined, *Dasyphyllum trichophyllum* (Baker) Cabrera (sect. *Macrocephala*), *D. brasiliense* (Spreng.) Cabrera, *D. spinescens* (Less.) Cabrera, and *D. tomentosum* (Spreng.) Cabrera (sect. *Microcephala*), all are trees. Stuessy et al. (1996) mentioned that the arborescent life form in *Dasyphyllum* might be a derived feature. *Schlechtendahlia*, all genera of Calyceraceae, and most of Goodeniaceae are herbaceous perennials. Stuessy et al. (1996) regarded *Schlechtendahlia* and *Acicarpha* as basal and took $x = 8$ as the original base number for both Asteraceae and Calyceraceae. Phylogenetic trees of Barnadesioideae by Bremer (1994) and Stuessy et al. (1996) are based on morphological characters and are influenced significantly by the life form. Molecular systematic data on the chloroplast *ndhF* sequences (Kim & Jansen, 1995) indicate that two clades including *Dasyphyllum* and *Schlechtendahlia* diverged at the base of subfamily Barnadesioideae. Gustaffson et al. (2001) and Funk et al. (2005) reported the more detailed molecular phylogenetic trees of the Barnasioides. Their trees show that the genera of *Barnadesia* and *Dasyphyllum* are at the basal position in the separate clades, and *Schlechtendahlia* is a derivative

in the *Dasyphyllum* clade. Both genera of *Barnadesia* and *Dasyphyllum* have a chromosome number of $2n = 54$, and *Schlechtendahlia* has a chromosome number of $2n = 16$. It is difficult to infer the dysploid reduction from $2n = 54$ to $2n = 18$ by the simple steps of chromosomal changes. Thus, we consider that the chromosome number of $n = 8$ for *Schlechtendahlia* has been derived from the ancestral $n = 9$ by the dysploid reduction, and the chromosome number of $2n = 54$ for *Barnadesia* and *Dasyphyllum* is a hexaploid state based on the original base chromosome number, $x = 9$. Because their genome sizes (= total haploid chromosome length) are so large (Table 1), some tribes have higher base chromosome numbers than previously suspected (i.e., $x = 17$ for Cardueae and Vernonieae, $x = 14$ for Gnaphalieae; Watanabe et al., 1999a). The base chromosome number in the clade comprising Heliantheae s.l.–Eupatorieae is $x = 18$. Estimated base chromosome numbers for helenioid tribes include $x = 19$ for Madieae, $x = 18$ for Helenieae s. str. and Perityleae, and $x = 17$ for Bahieae (Baldwin et al., 2002). Dysploid reduction in chromosome number from the putatively high base number in Heliantheae s.l.–Eupatorieae (Watanabe et al., 1995; Ito et al., 2000) and other tribes, except for Astereae ($x = 9$), Anthemideae ($x = 9$), Inuleae ($x = 11$–7), Lactuceae ($x = 9$), and Plucheeae ($x = 10$), to the typical diploid levels appears to have occurred repeatedly in different sublineages, especially in groups rich in annuals (Stebbins, 1950; Robinson et al., 1981; Kadereit & Jeffrey, 1996; Watanabe et al., 1996, 1999; Baldwin et al., 2002).

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APPENDIX 1. New chromosome number counts and voucher data for species of Asteraceae and Calyceraceae. *First report for species, **first report for genus.

CALYCERACEAE

Acicarpha spathulata R. Br. $2n = 8$II. Brazil. Sao Paulo: Itanhaem, Nova Itanhaem, *Watanabe & Nagatani 205* (GHSP, KYO).

ASTERACEAE

Barnadesieae

**Barnadesia caryophylla* (Vell.) S. F. Blake. $2n = 54$. Brazil. Goias: 11 km SW from Colinas do Sul to Niquelandia, *Watanabe & Nagatani 232* (GHSP, KYO); $2n = 54$. Brazil. Goiás: 13 km SW from Colinas do Sul to Niquelandia, *Watanabe & Nagatani 234* (GHSP, KYO).

***Dasyphyllum brasiliense* (Spreng.) Cabrera var. *brasiliense*. $2n = 54$. Brazil. Minas Gerais: 13 km SE from Carandai, *Watanabe & Nagatani 43 & 46* (GHSP, KYO); $2n = 54$. Brazil. São Paulo: cultivated in Jardim Botanico do Estado de São Paulo, *Watanabe & Nagatani 258* (GHSP, KYO).

***Dasyphyllum brasiliense* (Spreng.) Cabrera var. *multiflorum* (Baker) Cabrera. $2n = 54$. Brazil. Paraná: 54 km NE from Curitiba, *Watanabe & Nagatani 133* (GHSP, KYO).

***Dasyphyllum flagellare* (Casar.) Cabrera. $2n = 54$. Brazil. Minas Gerais: ca. 13 km from Corrego do Bom Jesus to Pedra de São Domingo, *Watanabe & Nagatani 109* (GHSP, KYO).

***Dasyphyllum spinescens* (Less.) Cabrera. $2n = 54$. Brazil. Minas Gerais: ca. 13 km from Corrego do Bom Jesus to Pedra de São Domingo, *Watanabe & Nagatani 108* (GHSP, KYO).

***Dasyphyllum tomentosum* (Spreng.) Cabrera var. *tomentosum*. $2n = 54$. Brazil. Santa Catarina: E Catanduvas along BR282, *Watanabe & Nagatani 144* (GHSP, KYO).

***Dasyphyllum tomentosum* (Spreng.) Cabrera var. *multiflorum* (Baker) Cabrera. $2n = 54$. Brazil. Santa Catarina: 14 km N from Santa Cecilia, *Watanabe & Nagatani 139* (GHSP, KYO).

***Dasyphyllum trichophyllum* (Baker) Cabrera. $2n = 54$. Brazil. Minas Gerais: São Tome das Letras, *Watanabe & Nagatani 96* (GHSP, KYO).

Mutisieae

**Chaptalia graminifolia* (Dusen) Cabrera. $2n = 48 + 0$–7Bs. Brazil. Paraná: waterfall of Mariquinha, 34 km from Ponta Grossa, *Watanabe & Nagatani 174* (GHSP, KYO).

Chaptalia integrifolia (Cass.) Baker. $2n = 48$. Brazil. São Paulo: 26 km SE from Cunha, *Watanabe & Nagatani 24* (GHSP, KYO). $2n = 48$. Brazil. Minas Gerais: 27 km SW from Barbacena to Ibertioga, *Watanabe & Nagatani 61* (GHSP, KYO); $2n = 48$. Brazil. Paraná: waterfall of Mariquinha, 34 km from Ponta Grossa, *Watanabe & Nagatani 177* (GHSP, KYO); $2n = 48$. Brazil. Santa Catarina: 13 km NE of São Joaquim, *Yahara et al. B317* (GHSP, KYO).

Chaptalia mandonii (Sch. Bip.) Burkart. $2n = 48$. Brazil. São Paulo: 26 km SE from Cunha, *Watanabe & Nagatani 25* (GHSP, KYO).

Chaptalia nutans (L.) Polak. $2n = 48$. Brazil. São Paulo: 17 km SE from Cunha to Parati, *Watanabe & Nagatani 8 & 9* (GHSP, KYO); $2n = 48$. Brazil. São Paulo: 26 km SE from Cunha, *Watanabe & Nagatani 23* (GHSP, KYO); $2n = 48$. Brazil. Santa Catarina: the W entrance of Campos Novos BR470, *Watanabe & Nagatani 142* (GHSP, KYO).

**Chaptalia runcinata* Kunth. $2n = 48$. Brazil. Paraná: waterfall of Mariquinha, 34 km from Ponta Grossa, *Watanabe & Nagatani 185* (GHSP, KYO).

**Jungia sellowii* Less. $2n = 20$II. Brazil. Santa Catarina: 13 km N of Urubici, *Yahara et al. B291* (GHSP, KYO).

**Mutisia campanulata* Less. $2n = 52$. Brazil. São Paulo: 3 km from Campode Jordao to Petra do Bau, *Watanabe & Nagatani 113* (GHSP, KYO); $2n = 52$. Brazil. São Paulo: ca. 10 km from Campos do Jordao to Santo Antonio do Pinhal through Lageado, *Watanabe & Nagatan 114* (GHSP, KYO).

Mutisia sp. $2n = 52$. Chile. *Ito 021501-2* (KANA).

***Stifftia chrysantha* Mikan var. *chrysantha*. $2n = 54$. Brazil. Rio de Janeiro: cultivated in Jardim Botanico do Estado de Rio de Janeiro, *Watanabe & Nagatani s.n.* (GHSP, KYO).

***Stifftia chrysantha* Mikan var. *fravicans* Toredo ex Dedecca. $2n = 54$. Brazil. São Paulo: cultivated in Jardim Botanico do Estado de São Paulo, *Watanabe & Nagatani 257* (GHSP, KYO).

**Trichocline catharinensis* Cabrera var. *catharinensis*. $2n = 38$. Brazil. Santa Catarina: 2.5 km SW of Lages, *Watanabe & Nagatani 146* (GHSP, KYO).

Trixis antimenorrhoea (Schrank) Mart. ex Baker. $2n = 54$. Brazil. Minas Gerais: 67 km SW from Barbacena, *Watanabe & Nagatani 72* (GHSP, KYO).

**Trixis praestans* (Vell.) Cabrera. $2n = 54$. Brazil. São Paulo: 18 km SE from Cunha to Parati, *Watanabe & Nagatani 11* (GHSP, KYO); $2n = 54$. Brazil. São Paulo: 26 km SE from Cunha, *Watanabe & Nagatani 28* (GHSP, KYO); $2n = 54$. Brazil. Minas Gerais: ca. 10 km from corrego do

Bom Jesus to Pedra de São Domingo, *Watanabe & Nagatani 104* (GHSP, KYO).

Vernonieae

**Eremanthus bicolor* (DC.) Baker. $2n = 30$. Brazil. Goiás: 14 km NE from Niquelandia, *Watanabe & Nagatani 236* (GHSP, KYO).

Eremanthus sp. 1. $2n = 30$. Brazil. Goiás: 15 km N of GO118 from Brasilia to Planaltina, *Watanabe & Nagatani 210* (GHSP, KYO).

Eremanthus sp. 2. $2n = 34$. Brazil. São Paulo: 2.5 km SE from Cunha to Parati, *Watanabe & Nagatani 15* (GHSP, KYO).

Eremanthus sp. 3. $2n = 17$II. Brazil. Rio de Janeiro: 4 km S of Petropolis, *Watanabe & Nagatani 35* (GHSP, KYO).

**Vernonia florida* Gardner. $2n = 34$. Brazil. Paraná: 3 km W of São Luis do Puruna, *Yahara et al. B208* (GHSP, KYO).

**Vernonia hypochaeris* DC. $2n = 17$II. Brazil. Santa Catarina: Morro do Quiriri, *Yahara et al. B134* (HGS, KYO, P); $2n = 34$. Brazil. Paraná: 3 km W of São Luis do Puruna, *Yahara et al. B235* (GHSP, KYO).

Vernonia nitidula Less. $2n = 17$II. Brazil. Santa Catarina: 10 km SW of Lages, *Yahara et al. B261* (GHSP, KYO).

Vernonia polyanthes Less. $2n = 34$. Brazil. São Paulo: 26 km SE from Guaratingueta to Cunha, *Watanabe & Nagatani 1* (GHSP, KYO); $2n = 34$+0–2Bs. Brazil. São Paulo: 26 km SE from Guaratingueta to Cunha, *Watanabe & Nagatani 3* (GHSP, KYO).

Vernonia scorpioides (Lam.) Pers. $2n = 34$. Brazil. Rio de Janeiro: Petropolis, 5 km S of Petropolis, *Watanabe & Nagatani 32* (GHSP, KYO); $2n = 17$II. Brazil. São Paulo: Nova Itanhaem, sandy beach, *Watanabe & Nagatani 206* (GHSP, KYO).

Vernonia sp. 1. $2n = 34$. Brazil. Goiás: 32 km W from Niquelandia to Uruacu, *Watanabe & Nagatani 237* (GHSP, KYO).

**Vernonia* sp. 2. $2n = 17$II. Brazil. Paraná: E limit of Guarapuava, *Yahara et al. B166* (GHSP, KYO).

Gnaphalieae

Achyrocline alata DC. $2n = 14$II. Brazil. Santa Catarina: 15 km N of Urubici. *Yahara et al. B302* (GHSP, KYO).

Achyrocline satureioides DC. $2n = 14$II. Brazil. Santa Catarina: 15 km N of Urubici, *Yahara et al. B303* (GHSP, KYO).

**Angianthus micropodioides* (Benth.) Benth. $2n = 12$II. Australia. W Australia: 2 km W of Hines Hill township, *Short 4449* (MEL, TI).

***Dithyrostegia amplexicaulis* A. Gray. $2n = 14$. Australia. W Australia: 12 km W of Pindar, *Short 4489* (MEL, TI).

***Epitriche demissus* (A. Gray) P. S. Short. $2n = 10$. Australia. W Australia: 5 km from Morawa on rd. to Perenjori, *Short 4508* (MEL, TI).

**Erymophyllum ramosum* subsp. *ramosum* (A. Gray) Paul G. Wilson. $2n = 12$II. Australia. W Australia: 138.9 km W from Balladonia, *Watanabe et al. 344* (MEL, TI).

Leptorhynchos squamatus (Labill.) Less. $2n = 12$II. Australia. Victoria: 36 km W of Mitre, *Watanabe et al. 303* (MEL, TI).

**Podotheca uniseta* P. S. Short. $2n = 26$. Australia. W Australia: 7 km S of Bunjil along rd. to Latham, *Short 4512* (MEL, TI).

Waitzia acuminata Steetz. $2n = 12$II. Australia. W Australia: 18 km S of Binnu, *Short 4492 & 4493* (MEL, TI).

Astereae

Baccharis anomala DC. $2n = 18$. Brazil. São Paulo: Cunha, 17 km SE from Cunha to Parati, *Watanabe & Nagatani 9* (GHSP, KYO); $2n = 9$II + BII. Brazil. Paraná: E of Guarapuava, *Yahara et al. B180* (GHSP, KYO); $2n = 9$II. Brazil. Santa Catarina: 13 km NE of São Joaquim, *Yahara et al. B310* (GHSP, KYO).

**Baccharis pentodonta* Malme. $2n = 9$II. Brazil. Paraná: 3 km W of São Luis do Puruna, *Yahara et al. B211* (GHSP, KYO).

**Baccharis triplinervis* Baker. $2n = 18$. Brazil. Santa Catarina: W entrance of Campos Novos BR470, *Watanabe & Nagatani 143* (GHSP, KYO).

Noticastrum acuminatum (DC.) Cuatrec. $2n = 18$. Brazil. Santa Catarina: 5 km S of Campo Alegre, *Yahara et al. B228* (GHSP, KYO); $2n = 9$II. Brazil. Santa Catarina: 10 km SW of Lages, *Yahara et al. B254* (GHSP, KYO).

Symphyotrichum graminifolium (Spreng.) G. L. Nesom. $2n = 18 + 0$–2Bs. Brazil. Santa Catarina: Serra da Anta Gorda, 13 km N of Urubici, *Yahara et al. B289* (GHSP, KYO).

Plucheeae

Pluchea sagittalis (Lam.) Cabrera. $2n = 10$II. Brazil. Santa Catarina: 10 km SW of Lages, *Yahara et al. B257* (GHSP, KYO).

Heleniеae

Chrysactinia truncata S. Watson. $2n = 30$. Mexico. Nuevo León: 3 km W from San Juan Bautista, *Yahara et al. 1458* (KYO, MEXU).

Dyssodia papposa (Vent.) Hitchc. $2n = 26$. Mexico. Coahuila: 15 km WNW of Coahuila–Nuevo Leon State border on Mex 57, *Yahara et al. 1467* (KYO, MEXU).

Dyssodia tagetiflora Lag. $2n = 26$. Mexico. Nuevo León: 29.4 km NW from Galeana, *Yahara et al. 1438* (KYO, MEXU).

Espejoa mexicana DC. $2n = 18$. Mexico. Guerrero: 1.5 km E of aeropuerto on Mex 200, *Yahara et al. 2001* (KYO, MEXU).

Florestina tripteris DC. $2n = 20$. Mexico. Nuevo León: 7.6 km E of Salida to General Bravo on hwy. 40D, *Yahaar et al. 1460* (KYO, MEXU).

Marshallia mohrii Beadle & F. E. Boynton. $2n = 36$. U.S.A. Missouri: *Yahara & Mishima s.n.* (KYO).

Palafoxia texana DC. $2n = 22$. Mexico. Veracruz: Playa palma Sola, *Yahara et al. 1365* (KYO, MEXU).

Porophyllum macrocephalum DC. $2n = 22$. Mexico. Oaxaca: Village of Sta. Maria Petapa, *Yahara et al. 1372* (KYO, MEXU).

Strotheria gypsophila B. L. Turner. $2n = 16$. Mexico. Nuevo León: 3 km W from Hwy. 57, salida to El Refugio, *Yahara et al. 142* (KYO, MEXU).

Thymophylla aurea (A. Gray) Greene. $2n = 16$. Mexico. Coahuila: 8.5 km N of Bermejillo, *Yahara et al. 1497* (KYO, MEXU).

Thymophylla gentryi (M. C. Johnst.) Strother. $2n = 16 + 0$–1B. Mexico. Coahuila: 15 km W of Mex 30 and Mex 45 jct., *Yahara et al. 1505* (KYO, MEXU).

Thymophylla pentachaeta (DC.) Small var. *pentachaeta*. $2n = 24$. Mexico. Nuevo León: 2.1 km SE of Zaragoza, *Yahara et al. 1414* (KYO, MEXU); $2n = 24$. Mexico. Coahuila: 15 km WNW of Coahuila–Nuevo León State border on Mex 57, *Yahara et al. 1466* (KYO, MEXU).

Thymophylla pentachaeta (DC.) Small var. *hartwegii* (A. Gray) Strother. $2n = 26$. Mexico. Nuevo León: 79 km from Monterrey to Sabinas Hidalgo on 85 Libre, *Yahara et al. 463* (KYO, MEXU).

Urbinella palmeri Greenm. $2n = 16$. Mexico. Durango: 49 km W of Durango City on Mex 40, *Yahara et al. 1507* (KYO, MEXU).

Heliantheae

Galinsoga parviflora Cav. $2n = 16II$. Brazil. Santa Catarina: 13 km N of Urubici, *Yahara et al. B299* (GHSP, KYO).

Jaegeria hirta (Lag.) Less. $2n = 18II$. Brazil. Santa Catarina: 13 km N of Urubici, *Yahara et al. B292* (GHSP, KYO).

Tithonia speciosa (Hook.) Hook. ex Griseb. 2n = 17II. Brazil. Minas Gerais: 4 km SW from Marbacena to Ibertiega, *Watanabe & Nagatani 52* (GHSP, KYO).

**Verbesina clausseni* Sch. Bip. ex Baker. 2n = 17II. Brazil. Santa Catarina: Morro do Quiriri, *Yahara et al. B233* (GHSP, KYO).

Eupatorieae

Ageratina cardiophylla (B. L. Rob.) R. M. King & H. Rob. $2n = 68$. Mexico. Michoacan: 9 km W from jct. to Zinapecuaro, *Yahara et al. 72* (KYO, MEXU).

**Ageratina hintonii* R. M. King & H. Rob. $2n = 34 + 0–2Bs$. Mexico. Guerrero: 64 km SW from Filo de Caballo. *Yahara et al. 54* (KYO, MEXU).

Ageratina sp. $2n = 34$. Mexico. Michoacan: 38 km E of Morelia, *Yahara et al. 73* (KYO, MEXU).

**Carphochaete wislizeni* A. Gray. $2n = 22$. Mexico. Chihuahua: 31.7 km W from Parral on Mex 24, *Yahara et al. 733* (KYO, MEXU).

**Chromolaena horminoides* DC. $2n = 30$. Brazil. Goiás: Serra dos Pirineus. 13 km W from Pirenopolis, *Watanabe & Nagatani 243* (GHSP, KYO).

**Chromolaena kleinii* (Cabrera) R. M. King & H. Rob. $2n = 10II$. Brazil. Santa Catarina: 13 km N of Urubici, *Yahara et al. B298* (GHSP, KYO).

Chromolaena laevigata (Lam.) R. M. King & H. Rob. $2n = 60$. Brazil. São Paulo: 21 km SE from Cunha to Pedra de Macela, *Watanabe & Nagatani 19* (GHSP, KYO).

**Chromolaena oinopolepis* (Malme) R. M. King & H. Rob. $2n = 10II + 10I$, $6III + 4II + 4I$. Brazil. Santa Catarina: 10 km SW of Lages, *Yahara et al. B281* (GHSP, KYO).

**Chromolaena pedunculosa* (Hook. & Arn.) R. M. King & H. Rob. $2n = 10II + 10I$, $8III + 2II + 2I$. Brazil. Paraná: Serra da Esperanca, *Yahara et al. B159* (GHSP, KYO); $2n = 20II + 10I$, $2n = 30$. Brazil. Santa Catarina: 10 km SW of Lages, *Yahara et al. B262 & B276* (GHSP, KYO).

Cronquistia pringlei (S. Watson) R. M. King. $2n = 24$. Mexico. Chihuahua: 95.7 km SW from Parral on Mex 24, *Yahara et al. 735* (KYO, MEXU).

**Decachaeta haenkeana* DC. $2n = 32$. Mexico. Guerrero: 24 km S of Chilpansingo, *Yahara et al. 60* (KYO, MEXU).

Erythradenia pyramidalis (B. L. Rob.) R. M. King & H. Rob. $2n = 32$. Mexico. Guerrero: 24 km S of Chilpansingo, *Yahara et al. 59* (KYO, MEXU).

Gyptis crassipes (Hieron.) R. M. King & H. Rob. $2n = 30$. Brazil. Santa Catarina: 10 km SW of Lages, *Yahara et al. B284* (GHSP, KYO).

Macvaughiella mexicana (Sch. Bip.) R. M. King & H. Rob. $2n = 24 + 4–8Bs$. Mexico. Oaxaca: 7 km SW of El Cameron, *Yahara & Soejima 372* (KYO, MEXU).

Microspermum debile Benth. $2n = 24$. Mexico. Guerrero: 64 km SW from Filo de Caballo, *Yahara et al. 53* (KYO, MEXU).

Microspermum nummulariaefolium Lag. $2n = 36$. Mexico. Guerrero: 14.8 km N of jct. to Ixtateopan, *Yahara & Soejima 239* (KYO, MEXU).

Mikania sp. $2n = 34$. Brazil. Minas Gerais: Conceicão do Ibitipoca, Serra de Ibitipoca, *Watanabe & Nagatani 84* (GHSP, KYO).

Oxylobus adscendens B. L. Rob. & Greenm. $2n = 32$. Mexico. Hidalgo: 8 km N from jct. to Mineral del Chico, *Yahara et al. 9* (KYO, MEXU).

**Praxelis missiona* (Malme) R. M. King & H. Rob. $2n = 80$. Brazil. São Paulo: 2 km N from Campos do Jordão, *Yahara et al. B128* (GHSP, KYO).

Piqueria pilosa Kunth. $2n = 24$. Mexico. Mexico: 29 km W of Jalatlaca, *Yahara et al. 90* (KYO, MEXU).

***Revealia macrocephala* (Paray) R. M. King & H. Rob. $2n = 22$. Mexico. Guerrero: 76 km SW from Filo de Caballo. *Yahara et al. 56* (KYO, MEXU).

**Stevia alternifolia* Hieron. $2n = 11II + 0–1B$, $2n = 22$. Brazil. Paraná: 3 km W of Palmeira, *Yahara et al. B190* (GHSP, KYO); $2n = 11II$. Brazil. Paraná: Rio dos Papagoios, *Yahara et al. B216* (GHSP, KYO).

**Stevia bangii* Rusby. $2n = 44 + 0–5Bs$. Bolivia. Laja: *Ito 010209-2* (KANA).

Stevia camachensis Hieron. $2n = 22$. Bolivia. Comarapa to Matara: *Ito ASA199-1* (KANA).

**Stevia cinerascens* Sch. Bip. ex Baker. $2n = 22$. Brazil. Santa Catarina: 2.5 km SW of Lages, *Yahara et al. B58* (GHSP, KYO); $2n = 22$. Brazil. Santa Catarina: 27 km S from Lages, *Yahara et al. B66 & B67* (GHSP, KYO); $2n = 11II$, $2n = 22$. Brazil. Santa Catarina: 10 km SW of Lages, *Yahara et al. B251 & B252* (GHSP, KYO).

**Stevia clausseni* Sch. Bip. ex Baker. $2n = 22$. Brazil. Santa Catarina: 14 km E of Bom Joaquim da Serra, *Yahara et al. B89* (GHSP, KYO); $2n = 22$. Brazil. Santa Catarina: 18 km E of Bom Joaquim da Serra, *Yahara et al. B99* (GHSP, KYO).

Stevia clinopodioides Greenm. $2n = 36 + 0–4Bs$. Mexico. Nuevo León: Cerro Potosi, *Yahara et al. 1440* (MEXU, KYO).

Stevia coahuilensis Soejima & Yahara. $2n = 33$. Mexico. Coahuila: 0.4 km E of Montereal, *Yahara et al 1668* (KYO, MEXU); $2n = 33$. Mexico. Coahuila: 9.4 km E of Montereal, *Yahara et al. 1696* (KYO, MEXU); $2n = 44$. Mexico. Nuevo León: 3.3 km W of Dieciocho de Marzo, *Yahara et al. 1794* (KYO, MEXU).

Stevia commixta B. L. Rob. $2n = 22$, $22,+2–3Bs$. Brazil. São Paulo: 2 km N of jct. from Campos do Jordao to Pedra do Bau, *Yahara et al. B1, B3 & B5* (GHSP, KYO); $2n = 22$. Brazil. São Paulo: 15 km N from Campos do Jordão to Pedra do Bau, *Yahara et al. B11 & B12* (GHSP, KYO).

Stevia decussata Baker. $2n = 22$. Brazil. Minas Gerais: Pico de São Domingo, *Yahara et al. B18* (GHSP, KYO).

Stevia hintoniorum B. L. Turner. $2n = 22$. Mexico. Coahuila: 5.2 km E of Montereal, *Yahara et al. 1695* (Type 1) (KYO, MEXU); $2n = 33$. Mexico. Coahuila: 0.4 km E of Montereal, *Yahara et al. 1665* (KYO, MEXU); $2n = 33 + 1B$. Mexico. Coahuila: 4.7 km E of Montereal, *Yahara et al. 1678* (KYO, MEXU); $2n = 44$. Mexico. Nuevo León: 17 km W of Dieciocho de Marzo, *Yahara et al. 1795 & 1796* (KYO, MEXU); $2n = 44 + 1B$. Mexico. Coahuila: 15.2 km E of Montereal, *Yahara et al. 1692* (Type 1) (KYO, MEXU).

Stevia incognita Grashoff. $2n = 33 + 1–3Bs$. Mexico. Coahuila: 11.3 km E of Montereal, *Yahara et al. 1704* (KYO, MEXU); $2n = 44 + 0–2Bs$. Mexico. Nuevo León: 17 km NE of San Antonio Pena Nevada, *Yahara et al. 1569* (KYO, MEXU).

Stevia jorullensis Kunth. $2n = 36$. Mexico. Nuevo León: 4.8 km W of San Ishidro, *Yahara et al. 1645* (KYO, MEXU); $2n = 36$. Mexico. Nuevo León: 5.8 km W of San

Ishidro, *Yahara et al. 1650* (KYO, MEXU); $2n = 36$. Mexico. Nuevo León: 15 km E of San Antonio de las Alazanas, *Yahara et al. 1662* (KYO, MEXU); $2n = 36$. Mexico. Coahuila: 10.1 km E of Jame, *Yahara et al. 1717* (KYO, MEXU); $2n = 36$. Mexico. Nuevo León: 11.6 km SE of Zaragoza, *Yahara et al. 1814* (KYO, MEXU); $2n = 36 + 0–1B$. Mexico. Nuevo León: 6.9 km SE of Zaragoza, *Yahara et al. 1808* (KYO, MEXU).

Stevia leptophylla Sch. Bip. ex Baker. $2n = 22 + 0–1B$. Brazil. Paraná: 6 km E of São Luis do Puruna, *Yahara et al. B151* (GHSP, KYO); $2n = 11II$. Brazil. Paraná: E limit of Lapa, *Yahara et al. B223* (GHSP, KYO).

**Stevia lundiana* DC. $2n = 22, 22 + 1B$. Brazil. São Paulo: 2 km N of Campos do Jordão to Pedra do Bau, *Yahara et al. B2 & B4* (GHSP, KYO); $2n = 22$. Brazil. Santa Catarina: 2.5 km S from Lages, *Yahara et al. B49* (GHSP, KYO); $2n = 22$. Brazil. Santa Catarina: 27 km S from Lages, *Yahara et al. B75* (GHSP, KYO); $2n = 11II$, $2n = 22$. Brazil. Santa Catarina: 15 km N of Urubici, *Yahara et al. B300* (GHSP, KYO); $2n = 22$. Brazil. Santa Catarina: 13 km NE of São Joaquim, *Yahara et al. B311* (GHSP, KYO).

**Stevia mitopoda* B. L. Rob. $2n = 24$. Mexico. Puebla: 21 km SE of Izcar de Matamoros on Mex 160, *Yahara et al. 1022* (KYO, MEXU).

**Stevia myriadenia* Sch. Bip. ex Baker. $2n = 11II$, $2n = 22$. Brazil. Santa Catarina: 15 km N of Urubici, *Yahara et al. B308 & B309* (GHSP, KYO).

Stevia nepetifolia Kunth. $2n = 33 + 3Bs$. Mexico. Coahuila: 0.4 km E of Montereal, *Yahara et al. 1667* (KYO, MEXU); $2n = 33$. Mexico. Coahuila: 4.7 km E of Montereal, *Yahara et al. 1669* (Type 2) (KYO, MEXU); $2n = 33 + 1B$. Mexico. Coahuila: 5.2 km E of Montereal, *Yahara et al. 1694* (Type 2) (KYO, MEXU); $2n = 44 + 1–4Bs$. Mexico. Nuevo León: 20 km NE from San Antonio Pena Nevada, *Yahara et al. 1596* (KYO, MEXU).

Stevia ophryophylla B. L. Rob. $2n = 11II$, $2n = 22$. Brazil. Paraná: E limit of Lapa, *Yahara et al. B220* (GHSP, KYO).

Stevia organensis Gardner. $2n = 22$. Brazil. São Paulo: Pedra do Bau, *Yahara et al. B8 & B148* (GHSP, KYO).

Stevia ovata Willd. $2n = 22 + 0–2Bs$. Mexico. Nuevo León: 6 km W of Cola de Caballo, *Yahara et al. 1619* (KYO, MEXU); $2n = 33 + 0–2Bs$. Mexico. Coahuila: 10.1 km E of Jame, *Yahara et al. 1715* (KYO, MEXU); $2n = 33 + 3Bs$. Mexico. Nuevo León: 69 km SE of Zaragoza, *Yahara et al. 1806* (KYO, MEXU); $2n = 33 + 2–3Bs$. Mexico. Coahuila: 5.2 km E of Montereal, *Yahara et al. 1693* (KYO, MEXU); $2n = 33 + 3–8Bs$. Mexico. Nuevo León: 8.2 km W of San Ishidro, *Yahara et al. 1654* (KYO, MEXU); $2n = 44 + 2Bs$. Mexico. Nuevo León: 20 km NE from San Antonio Pena Nevada, *Yahara et al. 1600* (KYO, MEXU).

**Stevia parvifolia* Hassl. $2n = 11II$, $2n = 22$. Brazil. Santa Catarina: Morro do Quiriri, *Yahara et al. B232* (GHSP, KYO).

Stevia pilosa Lag. $2n = 33$. Mexico. Nuevo León: 3.3 km W of Dieciocho de Marzo, *Yahara et al. 1786* (KYO, MEXU); $2n = 33$. Mexico. Coahuila: 15 km E from San Antonio de las Alazanas, *Yahara et al. 1663* (KYO, MEXU); $2n = 33 + 0–1B$. Mexico. Nuevo León: 20 km NE from San Antonio Pena Nevada, *Yahara et al. 1576* (KYO, MEXU); $2n = 44$. Mexico. Coahuila: 1.7 km E of Montereal, *Yahara et al. 1679* (KYO, MEXU); $2n = 44$. Mexico. Coahuila: 15.2 km E of Montereal, *Yahara et al. 1691* (KYO, MEXU).

Stevia porphyrea McVaugh. $2n = 44$. Mexico. Nuevo León: 1 km W of San Ishidro, *Yahara et al. 1640* (KYO, MEXU).

Stevia potosina Soejima Yahara & K. Watan. $2n = 33$. Mexico. Nuevo León: 15 km E of San Antonio de las Alazanas, *Yahara et al. 1661* (KYO, MEXU); $2n = 33$. Mexico. Nuevo León: 3.3 km W of Dieciocho de Marzo, *Yahara et al. 1680* (KYO, MEXU); $2n = 33$. Mexico. Nuevo León: 12 km W of Dieciocho de Marzo, *Yahara et al. 1776* (KYO, MEXU); $2n = 33$. Mexico. Nuevo León: 12 km W of Dieciocho de Marzo, *Yahara et al. 1800* (KYO, MEXU); $2n = 33 + 0–1B$. Mexico. Coahuila: 10.1 km E of Jame, *Yahara et al. 1716* (KYO, MEXU).

Stevia purpusii B. L. Rob. $2n = 36$. Mexico. Morelos: Steep mtn. above Tepoztlan, *Yahara et al. 2029* (KYO, MEXU).

Stevia selloi Sch. Bip. ex Baker. $2n = 22$. Brazil. Santa Catarina: 2.5 km SW of Lagas, *Yahara et al. B51* (GHSP, KYO); $2n = 22$. Brazil. Santa Catarina: 27 km S from Lagas, *Yahara et al. B65* (GHSP, KYO); $2n = 11II$. Brazil. Paraná: 3 km W of Palmeira, *Yahara et al. B191* (GHSP, KYO).

Stevia serrata Cav. var. *serrata*. $2n = 44$. Mexico. Coahuila: 1.7 km E of Montereal, *Yahara et al. 1671* (KYO, MEXU); $2n = 44 + 1B$. Mexico. Coahuila: 4.7 km E of Montereal, *Yahara et al. 1670* (KYO, MEXU); $2n = 44 + 1–2Bs$. Mexico. Nuevo León: 20 km NE from San Antonio Pena Nevada, *Yahara et al. 1585* (KYO, MEXU).

Stevia setifera Rusby ex B. L. Rob. $2n = 22$. Bolivia. Unduavi: *Ito 15-2st* (KANA).

Stevia sp. 2. $2n = 22$. Bolivia. Sta Cruz New Rd., near Siberia, *Ito 0102095-2* (KANA).

**Stevia tenuis* Hook. & Arn. $2n = 22$. Brazil. Santa Catarina: 14 km E of Bom Joaquim da Serra, *Yahara et al. B97* (GHSP, KYO).

Stevia tephra B. L. Rob. $2n = 33 + 1–2Bs$. Mexico. Nuevo León: 1 km W of San Ishidro, *Yahara et al. 1640* (KYO, MEXU); $2n = 33 + 3Bs$. Mexico. Nuevo León: 8.2 km W of San Ishidro, *Yahara et al. 1656* (KYO, MEXU); $2n = 33 + 0–1B$. Mexico. Nuevo León: 45.4 km SW of Mex 58 & 85 jct. in Linares, *Yahara et al. 1771* (KYO, MEXU); $2n = 33 + 3–4Bs$. Mexico. Nuevo León: 45.4 km SW of Mex 58 & 85 jct. in Linares, *Yahara et al. 1772* (KYO, MEXU).

Stevia tomentosa Kunth. $2n = 33 + 2Bs$. Mexico. Nuevo León: 45.4 km SW of Mex 58 & 85 jct. in Linares, *Yahara et al. 1770* (KYO, MEXU); $2n = 33 + 3Bs$. Mexico. Nuevo León: 8.2 km W of San Ishidro, *Yahara et al. 1651* (KYO, MEXU); $2n = 44 + 2–4Bs$. Mexico. Nuevo León: 8.7 km SE of Zaragoza, *Yahara et al. 1811* (KYO, MEXU).

Stevia veronicae DC. $2n = 11II$. Brazil. Paraná: E limit of Guarapuava, *Yahara et al. B170* (GHSP, KYO); $2n = 11II$, $2n = 22$. Brazil. Paraná: 3 km W of São Luis do Puruna, *Yahara et al. B204 & B205* (GHSP, KYO); $2n = 11II$. Brazil. Santa Catarina: 10 km SW of Lages, *Yahara et al. B250* (GHSP, KYO).

SYSTEMATICS OF THE GENUS *MENTZELIA* (LOASACEAE) IN SOUTH AMERICA[1]

Maximilian Weigend[2]

Abstract

South American taxa of *Mentzelia* L. (Loasaceae) are revised and morphologically and ecologically characterized on the basis of extensive field studies, cultivation of several taxa, and a revision of over 1000 herbarium specimens (over 550 collections). On the basis of this study, a total of 14 taxa in eight species and six subspecies are recognized: *M. albescens* (Gillies ex Arn.) Griseb., *M. aspera* L., *M. bartonioides* (C. Presl) Urb. & Gilg, *M. heterosepala* Weigend & E. Rodr., *M. parvifolia* Urb. & Gilg ex Kurtz, and *M. scabra* Kunth, plus the two new species *M. sericea* Weigend (northern Peru) and *M. angurate* Weigend (central Peru). The latter are segregated from *M. scabra*, which is redefined. *Mentzelia scabra* is subdivided into six subspecies: one represents the autonym (subsp. *scabra*, Colombia), four are new status and combinations (subsp. *cordobensis* (Urb. & Gilg ex Kurtz) Weigend [Argentina], subsp. *grandiflora* (Ruiz & Pav.) Weigend [coastal Peru], subsp. *atacamensis* (Urb. & Gilg) Weigend [northern Chile], subsp. *chilensis* (Gay) Weigend [Venezuela, Colombia, Ecuador, Peru, Chile, Bolivia]), and one is a new subspecies (subsp. *boliviana* Weigend [Bolivia]). Lectotypes are designated for *M. andina* Urb. & Gilg, *M. cordifolia* Dombey ex Urb. & Gilg, *M. fragilis* Huber, *M. grisebachii* Urb. & Gilg, *M. parvifolia*, *M. scabra* subsp. *atacamensis*, *M. scabra* subsp. *chilensis*, *M. soratensis* Urb. & Gilg, and *M. scabra* subsp. *cordobensis*. The following taxa are newly synonymized: *M. pinnatifida* (Phil.) Urb. & Gilg (= *M. bartonioides*), *M. jujuyensis* Sleumer and *M. parvifolia* var. *transiens* Sleumer (= *M. parvifolia*), *M. cordifolia* (= *M. scabra* subsp. *grandiflora*), and *M. fendleriana* Urb. & Gilg, *M. ignea* (Phil.) Urb. & Gilg, and *M. soratensis* (= *M. scabra* subsp. *chilensis*). Taxon delimitations in *M. scabra* and its allies have traditionally been based on stamen number, petiole length, petal length, leaf subdivision, seed morphology, and density of trichome cover on stem, all of which are of limited systematic usefulness. Taxa are redefined here primarily on the basis of trichome characters on the ovary, a rather conservative (within and between populations) and readily observed character complex, and additionally on the basis of growth habit, presence or absence of persistent bark, and, at an infraspecific level, fruit shape and petal length. *Mentzelia parvifolia* from southern Peru is reported here for the first time.

Key words: Andes, Loasaceae, *Mentzelia*, South America.

The genus *Mentzelia* L. is the second largest genus of the family Loasaceae, comprising over 80 species (the largest genus is primarily South American *Nasa* Weigend, with ca. 100 species; Weigend, 2004). *Mentzelia* is widespread in the Americas, but has its center of diversity in the southwestern part of North America. Only a single weedy species is found outside the New World: *M. aspera* L. is an introduced weed, e.g., on the Cape Verde Islands (Pinto-Basto, 1995). The vast majority of species are from semi-desert habitats and dry scrub, with only very few taxa entering more permanently humid habitats.

There have been several complete revisions of the genus (Urban & Gilg, 1900; Darlington, 1934), some revisions for particular geographical regions (Macbride, 1941; Sleumer, 1955; Ernst & Thompson, 1963; Weigend, 2000, 2001), and descriptions of individual taxa from the United States and Mexico (e.g., Thompson & Zavortnik, 1968; Thompson & Roberts, 1971; Turner & Hempel, 1995; Holmgren & Holmgren, 2002; Reveal, 2002;), but only two species have been described from South America (Sleumer, 1955; Weigend & Rodriguez, 1999) since the revision by Urban and Gilg (1900). While a total of ca. 200 species and infraspecific names have been published in *Mentzelia*, many of them have since been reduced to synonymy, including many names originally published by Urban and Gilg (1900). There is

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scattered information on various aspects of the morphology, anatomy, and floral biology of *Mentzelia* (Urban, 1892; Gilg, 1925; Booth, 1933; Leins & Winhard, 1973; Hill, 1976; Brown & Kaul, 1981; Keeler, 1981; Eisner et al., 1998; Spackman Panjabi, 2004), but none of these studies deal with South American taxa. *Mentzelia* is subdivided into a total of four sections, all of which are found in North America and three of which, *Mentzelia*, *Trachyphytum* Torr. & A. Gray, and *Bartonia* Torr. & A. Gray, have native representatives in South America (Darlington, 1934). The genus *Mentzelia* has been the subject of two phylogenetic studies (Hill, 1976; Hufford et al., 2003), both of which largely retrieve the sections recognized by Urban and Gilg (1900) and Darlington (1936) as natural groups. *Mentzelia* section *Mentzelia*, the most speciose group in South America (eight species are recognized here), appears to be one of the two basal lineages, whereas the other two sections present in South America (*Bartonia* and *Trachyphytum*) are among the more derived groups in the genus and clearly have their center of diversification in North America. Section *Bartonia* is represented by a single biennial, herbaceous species in South America, *M. albescens* (Gillies ex Arn.) Griseb., which is also found in North America. Section *Trachyphytum* includes some four annual species from Chile and Argentina (Urban & Gilg, 1900; Darlington, 1934). *Mentzelia* sect. *Mentzelia* is the largest group of *Mentzelia* in South America with a total of 28 species and infraspecific names published. The two previous complete revisions of the genus *Mentzelia* were based on very little material of *Mentzelia* in South America: Urban and Gilg (1900) had seen eight collections of *Mentzelia* sect. *Trachyphytum* and divided these into four species, whereas Darlington (1934) had seen one specimen and three photographs of specimens and recognized three species. Similarly, Urban and Gilg (1900) and Darlington (1934) had seen only 44 and 40 specimens, respectively, of the *Mentzelia hispida* Willd. group, in which they recognized 10 and nine taxa, respectively. Neither of the two studies included any observations in the field or of living plants in cultivation. The morphological differences that have been used in identifying species in South America are mainly seed morphology (winged seed vs. wingless seed), petiole length (lamina sessile vs. distinctly petiolate), number of anthers (70 to 80 vs. 100 to 120), leaf dissection (deeply lobed vs. subentire; Urban & Gilg, 1900), and petal length (Darlington, 1934). The present study focuses on investigating taxon limits on the basis of field observation, cultivated plants, and the revision of herbarium specimens to verify which, if any, of these characters are systematically useful in defining taxa. The classification provided here should be seen as a broadly based morphological hypothesis inviting further studies, especially those using molecular techniques, to elucidate the phylogeny of the group. The revised classification proposed in the following descriptions recognizes only eight South American species, one each in *Mentzelia* sect. *Bartonia* (*M. albescens*) and *Mentzelia* sect. *Trachyphytum* (*M. bartonioides* (C. Presl) Urb. & Gilg), and six in *Mentzelia* sect. *Mentzelia* (*M. angurate* Weigend [new species], *M. aspera*, *M. heterosepala* Weigend & E. Rodr., *M. parvifolia* Urb. & Gilg ex Kurtz, *M. scabra* Kunth, and *M. sericea* Weigend [new species]). A total of six subspecies are recognized in *M. scabra* (subsp. *atacamensis* (Urb. & Gilg) Weigend [new status and combination], subsp. *boliviania* Weigend [new subspecies], subsp. *chilensis* (Gay) Weigend [new status and combination], subsp. *cordobensis* (Urb. & Gilg ex Kurtz) Weigend [new status and combination], subsp. *grandiflora* (Ruiz & Pav. ex G. Don) Weigend [new status and combination]; and subsp. *scabra*).

Material and Methods

Plant material and observations were derived from a total of 11 field trips to South America (Argentina, Chile, Colombia, Ecuador, Peru, and Venezuela). These led to over 60 collections of *Mentzelia*, typically including seed material, formalin acetic alcohol–preserved material, and photographs. Field observations were made on seven of the eight species recognized here and on four of the five subspecies of *M. scabra*. Over 1000 herbarium specimens (over 550 collections) of *Mentzelia* from South America were revised, including most extant nomenclatural types from the following 39 herbaria: AA, B, BM, BR, CEPEC, COL, CPUN, CUZ, E, F, FR, G, GB, GOET, GUAY, HAO, HUSA, HUT, K, LOJA, LPB, M, MO, MOL, MSB, NY, P, PSO, QCA, QCNE, S, UC, UMSA, US, USM, W, WIS, WU, Z. Six different seed lots of three taxa (*M. heterosepala*, *M. sericea*, *M. scabra* subsp. *chilensis*) were germinated and cultivated until fruit maturity in the green houses at Berlin.

Microscopy

For scanning electron microscopy (SEM), the plant material was either mounted directly (seeds, pollen) or critical point–dried after dehydration in a graded alcohol series following the standard procedure (flowers, fruits). Material was then sputter-coated with gold and subsequently viewed and photographed in an LEO VP 438 scanning electron microscope (Leo Electron Microscopy Ltd., Cambridge, United Kingdom) at a voltage of 15 kV.

Results

Morphology

Most South American *Mentzelia* species are caulescent plants that already have well-spaced leaves in the seedling stage; only *M. bartonioides* and *M. albescens* have a distinct basal leaf rosette. *Mentzelia bartonioides*, *M. aspera*, and *M. parvifolia* are annuals, and *M. albescens* is biennial. *Mentzelia sericea*, *M. angurate*, and the subspecies of *M. scabra* are typically subshrubs, but can flower and fruit in the first season (i.e., facultatively annuals). *Mentzelia heterosepala* and *M. scabra* subsp. *scabra* are clearly shrubs, a growth habit also occasionally found in *M. scabra* subsp. *chilensis* and *M. sericea*. The flowers of *Mentzelia* sect. *Mentzelia* are always heterochlamydeous and pentamerous. The sepals are shorter than the petals, narrowly ovate, and usually long-acuminate and more or less homogeneous. Only *M. heterosepala* has two larger outer sepals, followed by one sepal of intermediate size and two narrower inner sepals. The petals are always widely ovate, obovate or subcircular, and acuminate. They are off-white to pale yellow in *M. albescens* and yellow to orange in all other South American species. The androecium consists of numerous anthers (20 to 120), but the exact number is not fixed and the primary flowers have distinctly more stamens than the secondary and higher-order flowers, which renders a species distinction on the basis of simple stamen number (Gilg, 1893; Urban & Gilg, 1900; Darlington, 1934) extremely problematic. While the maximum number of stamens differs between *M. scabra* subsp. *scabra* and *M. scabra* subsp. *chilensis*, there is broad overlap between late flowers of the former and primary flowers of the latter. Stamen number is therefore not used here as a systematic criterion. Variability of stamen numbers has already been documented by Brown and Kaul (1981) for other species in *Mentzelia*.

Some degree of heteranthery is nearly universally present in *Mentzelia*, ranging from slight size differences (outer and especially antesepalous stamens with longer filaments) to increasingly divergent outer/antesepalous filamens (Urban, 1886, 1892; Urban & Gilg, 1900; Darlington, 1934; Brown & Kaul, 1981). In *M. bartonioides*, the antesepalous filaments are often distinctly widened in their proximal half, and this has been used to distinguish between species by Urban and Gilg (1900), but has been shown to be inconsistent within and between individuals by Sleumer (1955), who consequently synonymized the species differentiated on the basis of this character. Similarly, *M. aspera* typically has antesepalous stamens with petaloid filaments, but many populations (Fig. 4A; *Weigend et al. 8421*, *8516*) have uniformly filiform filaments; thus, this character seems to be useless for the purposes of classification. In *M. heterosepala*, *M. scabra*, *M. angurate*, and *M. sericea*, the ca. 10 outer filaments are usually much longer (1.5–2×) than the inner filaments, such that an outer series of anthers distinctly overtops the inner anthers, which, in turn, form a more or less tight fascicle in the center of the flower, at least in early anthesis. This phenomenon was first illustrated in Ruiz and Pavón (1958: 457, "*Mentzelia fulva*" = *M. scabra* subsp. *grandiflora*) and is also found in many other species of *Mentzelia* sect. *Mentzelia* outside South America (U.S.A.: *M. oligosperma* Nutt., *N.C. Henderson 63-972* (FR); *M. floridana* Nutt., *A. H. Curtiss 959* (FR); and Mexico: *M. hispida* Willd., *W. Boege 1445* (FR)). Loasaceae usually have ring nectaries or antesepalous groups of secretory stomata (Thompson & Ernst, 1967; Brown & Kaul, 1981; Weigend, 1997). Ring nectaries are present in *M. albescens*, as has been previously reported (Urban, 1886, 1892), but nectaries and nectar production are absent in all taxa of *Mentzelia* sect. *Mentzelia* (*M. aspera*, *M. angurate*, *M. heterosepala*, *M. parvifolia*, *M. scabra*, and *M. sericea*) in South America. Pollen is the only pollinator reward.

The capsules of *Mentzelia* sect. *Mentzelia* are sessile to shortly pedicellate. Fruit dehiscence is usually by three apical valves, but additionally, the calyx and sometimes the entire ovary roof are often shed at maturity in *M. aspera* and *M. parvifolia*. In these latter taxa, the seeds usually fail to fall out of the capsule through the small apical opening, but remain trapped in the fruit until the capsule wall disintegrates. Otherwise dehiscence is reversibly xerochasious, as is typical of most Loasaceae (Weigend et al., 2004). The fruits remain firmly attached to pedicel and plant in *M. albescens*, *M. aspera*, *M. bartonioides*, *M. heterosepala*, and *M. parvifolia*, but the fruit can also break off when they come into contact with the fur/clothing of a passing mammal and are then dispersed as a unit in some taxa (e.g., *M. scabra* and subspecies).

Scabrid glochidiate trichomes come in many shapes in Loasaceae and are often good taxonomic markers (Dostert & Weigend, 1999). In *Mentzelia* the entire above-ground parts of the plant (with the exception of the petals, stamens, style, and sometimes the ovary roof) are covered with these trichomes. In *Mentzelia* sect. *Mentzelia*, the indumentum of the fruits is one of the very few characters that is stable not only within populations, but also in groups of populations, and correlates well with geographical distribution, as well as with habit and (often) leaf dissection. Moreover, the characters are readily defined and observed with a hand lens, so they appear ideally suited for taxon

delimation. Individual trichomes consist of an apical unicellular trichome-cell and a group of five to 18 basal cells that are often elevated above the epidermis, especially in large trichomes (Fig. 1C, D). The distinction between scabrid and glochidiate trichomes depends on the nature of the surface sculpturing. If sculpturing is blunt or acroscopically curved and relatively short (less than shaft diameter, Fig. 1C), the trichomes are termed scabrid, and in this case, the trichome apex is usually tapering and smooth. If the sculpturing is basiscopically curved and longer (at least some equaling the shaft diameter, Fig. 1D), the trichomes are called glochidiate. Typically, the glochidiate trichomes have a ring of long projections at the tip, giving them the appearance of an anchor, which is why the term "ankerhaare" (anchor trichomes) has been used in the literature (Fig. 4B–D; Solereder, 1899). All parts of the plants are covered with scabrid and/or glochidiate trichomes, but only a comparison for the outside of the ovary/capsule is illustrated here. The differences here are particularly striking and more clearly observed than on the leaves and stem, and are therefore used in taxon delimitation in *Mentzelia* sect. *Mentzelia*.

Seeds. The seed morphology of *Mentzelia* in North America has been studied in some detail by Hill (1976), who showed that seed morphology largely supports the infrageneric groups proposed by Urban and Gilg (1900) and Darlington (1934). The seeds of *M. albescens* (Fig. 1E, F) are very similar to those of other taxa in section *Bartonia*, as are those of *M. bartonioides* (Fig. 5D, E) to other taxa of section *Trachyphytum* illustrated in Hill (1976). The seeds of the taxa of *Mentzelia* sect. *Mentzelia* studied here (*M. aspera*: Fig. 4E, F; *M. angurate*, *M. heterosepala*: Fig. 6D–F; *M. parvifolia*: Fig. 8E, F; and *M. sericea*: Fig. 17D, E) are irregularly oblique-ovoidal, laterally compressed, and have irregularly oblong testa epidermis cells with shallow (e.g., *M. scabra*, *M. heterosepala*) or deep (e.g., *M. parvifolia*) transverse (equatorial) grooves or striations. This seed morphology corresponds closely to that of North American taxa of *Mentzelia* sect. *Mentzelia* (Hill, 1976). The winged seeds used to distinguish taxa by Urban and Gilg (1900) are an artifact: immature seeds collapse strongly and then appear to have a narrow longitudinal wing, while none of the taxa have winged seeds when mature.

Taxonomic Treatment

Key to *Mentzelia* in South America

1a. Erect, annual or biennial herbs with distinct basal leaf rosette (usually withered when plant is in flower), leaves narrowly obovate to oblong, sinuate to pinnatifid.
  2a. Annual herbs; flowers with 5 (small) petals; seeds irregularly and sharply angular (sect. *Trachyphytum*) . . . . . 4. *M. bartonioides*
  2b. Biennial herbs; flowers seemingly with 10 petals (5 petals and 5 petaloid staminodia); seeds flat and disc-shaped with wide marginal wing (sect. *Bartonia*) . . . . . 1. *M. albescens*
1b. Laxly branched herbs, shrubs or subshrubs with long, leafy stems, basal leaf rosette never present; leaves (widely) ovate, typically with 1 large leaf lobe on each side, rarely leaves subentire . . . . . *Mentzelia* sect. *Mentzelia*
  3a. Annual herbs; ovary covered with glochidiate trichomes only; bracts of terminal flowers recaulescent on axillary branches (when present), sometimes to the next flower; capsule cylindrical to narrowly clavate, sessile or subsessile with barely narrowed base.
    4a. Inflorescence branches divergent with an angle of ca. 90 ; leaves with 1 distinct lobe near the base on each side, margin ± regularly serrate; mature capsules 18–25 mm long, narrowed at base . . . . . 3. *M. aspera*
    4b. Inflorescence branches divergent with an angle of ca. 160 –180 ; leaves indistinctly lobed, sometimes pinnately lobed, sometimes with a single lobe on each side, that lobe then with a distinct proximal lobule, margin irregularly serrate to lobulate; mature capsules 8–14 mm long, not narrowed at base but often somewhat narrowed ca. 1/3 from base . . . . . 6. *M. parvifolia*
  3b. Shrubs or subshrubs, rarely annual shrubs; ovary covered with both scabrid and glochidiate trichomes, or with scabrid trichomes only; bracts not or only slightly recaulescent; capsule subglobose to (rarely) widely clavate, pedicellate, subglobose if subsessile.
    5a. Leaves subentire or entire; sepals distinctly different in width; inflorescence few-flowered with 1 to 3 flowers, rarely many-flowered (4 to 10) and compact (subterminal branches only slightly overtopping primary flower) . . . . . 5. *M. heterosepala*
    5b. Leaves always serrate and usually lobed; sepals ± identical in width (outer ones at most 1.5× as wide as inner ones); inflorescence many-flowered (> 20), diffuse, subterminal branches far overtopping primary flower.
      6a. Indument of ovary consisting of scabrid and glochidiate trichomes of widely differing lengths . . 7. *M. scabra*
      6b. Indument of ovary consisting only of scabrid trichomes of similar lengths.

7a. Ovaries and capsules widely ovoidal to subglobose, densely covered with yellow, acroscopic to half-erect, scabrid trichomes 1.5–2 mm long; decumbent to ascending subshrub . . 2. *M. angurate*

7b. Ovaries and capsules conical to narrowly ovoidal, densely covered with white, appressed scabrid trichomes up to 0.5 mm long; stiffly erect subshrub or shrub . . . . . 8. *M. sericea*

Mentzelia L., Sp. Pl. 1: 516. 1753. TYPE: *Mentzelia aspera* L., Sp. Pl. 1: 516. 1753.

Bartonia Pursh ex Sims, Bot. Mag. 36: t. 1487. 1812, nom. illeg., non *Bartonia* Mühl. ex Willd., Gentianaceae, 1801. *Nuttallia* Raf., Amer. Monthly Mag. 1: 358. 1817. *Hesperaster* Cockerell, Torreya 1: 142. 1901. nom. illeg. TYPE: *Bartonia decapetala* Pursh ex Sims [= *Mentzelia decapetala* (Pursh ex Sims) Urb. & Gilg].

Torreya Eaton, Man. Bot., ed. 5: 420–421. 1829, nom. rej., non *Torreya* Arn., Taxaceae, 1838, nom. cons. TYPE: *Torreya ornata* (Pursh ex Sims) Eaton, nom. illeg. [= *Mentzelia ornata* (Pursh ex Sims) Torr. & A. Gray].

Acrolasia C. Presl, Reliq. Haenk. 2: 39. 1831. TYPE: *Acrolasia bartonioides* C. Presl [= *Mentzelia bartonioides* (C. Presl) Urb. & Gilg in Engl. & Prantl].

Creolobus Lilja, Fl. Sv. Odl. Vext.: 67. 1839. *Chrysostoma* Lilja, Fl. Sv. Odl. Vext. Suppl. 1: 33. 1840, nom illeg. TYPE: *Creolobus aureus* Lilja.

Bicuspidaria Rydb., Bull. Torrey Bot. Club 30: 275. 1903. TYPE: *Bicuspidaria tricuspis* (A. Gray) Rydb.

Annual or perennial herbs, subshrubs, shrubs, or small trees with scabrid-glochidiate trichomes, without stinging hairs, dominant and persistent taproot present. Leaves sessile or petiolate, opposite below and alternate above, rarely opposite or alternate throughout; lamina ovate to triangular-ovate, occasionally lyrate, usually lobed, margin serrate or mucronate. Inflorescences complex, terminal, basitonic thyrsoids, frondose, bracteose or frondose-bracteose; each flower with 2 frondose prophylls; sepals green, triangular or ovate, acuminate, margin entire; flowers with inferior ovary, calyx lobes ovate, acuminate or apiculate, white, yellow, or orange; stamens 10–120, filaments equal to very unequal in size, sometimes outer filaments dilated or apically forked, or petaloid staminodes; style 1, filiform, stigmatic surfaces long-decurrent on style. Capsule inferior, sessile or pedicellate, opening with 3 to 7 apical valves; seeds angular or laterally compressed; testa reticulate or polyhedral, sometimes with central papillae, sometimes narrowly winged.

Distribution and habitat. Ca. 80 species, from Argentina to Canada including the Caribbean and Galápagos Islands, most in the southwestern U.S.A. and Mexico. The majority of species are in desert and semi-desert habitats, some penetrating into grasslands and tropical dry forests.

1. Mentzelia albescens (Gillies ex Arn.) Griseb., Abh. Königl. Ges. Wiss. Göttingen Math.-Phys. Kl. 19: 150. 1874. Basionym: *Bartonia albescens* Gillies ex Arn., Edinburgh J. Nat. Geogr. Sci. 3: 273. 1831. TYPE: Argentina. Mendoza, *J. Gillies s.n.* (holotype, BM!; isotypes, E!, K not seen). Figure 1.

Bartonia sinuata C. Presl, Reliq. Haenk. 2: 38. 1831. TYPE: Chile. *T. P. X. Haenke s.n.* (type, PR not seen).

Biennial herb 0.1–0.85 m tall, forming a dense leaf rosette in the first year and a much-branched, stiffly erect flowering shoot in the second year; stems basally 5–15 mm thick, shoot densely branched, with a single, little-branched taproot; stem off-white, densely covered with glochidiate trichomes. Leaves subsessile with long-tapering base, basal leaves numerous in dense, biennial rosette, upper ones alternate; lamina narrowly obovate, 25–200 × 5–35 mm, sinuate-dentate with rounded lobules; base long-tapering; apex rounded to acuminate. Inflorescences frondose-bracteose, many-flowered (> 15), strongly basitonic, dense and brush-like (branching angle between paraclades of dichasia mostly < 60°); proximal internodes 1–3× as long as subtending bracts, distal internodes much shorter than subtending bracts: bracts ovate, entire to irregularly dentate, 5–50 × 5–25 mm; flowers subsessile. Flowers with calyx tube cylindrical from conical base, 6.5–13 × 3.5–5 mm, densely covered with erect, thin, glochidiate trichomes 0.1–0.6 mm long; calyx lobes 5–6 × 2–3.5 mm, ovate-acuminate; petals widely ovate, 6.5–10 × 2.3–3.5 mm, apex acuminate to apiculate, off-white to pale yellow; petaloid stamens 5, antesepalous, similar in shape, size, and color as petals; fertile stamens 40 to 60, filaments 3–7 mm, outer ones longer than inner ones, anthers ca. 0.6 × 0.4 mm; style filiform, 5.5–7 mm, with 3 decurrent stigmas. Capsule sessile, cylindrical, 5–10 × 3–5 mm, opening with 3 apical valves; seeds 70 to 110, ca. 2.5–3.5 mm wide, flat, disc-shaped with circular wing, white to off-white.

Mentzelia albescens is the only known biennial species of the genus in South America, where it is restricted to Chile and Argentina. It is readily recognized by its flowers that appear to have 10 petals (five petals and five petaloid staminodia), and it is the only South American species of *Mentzelia* sect. *Bartonia*, which has ca. 30 species in North America (Darlington, 1934). *Mentzelia albescens* itself is also found in North America (southern U.S.A., Mexico; Perez-Moreau & Crespo, 1988).

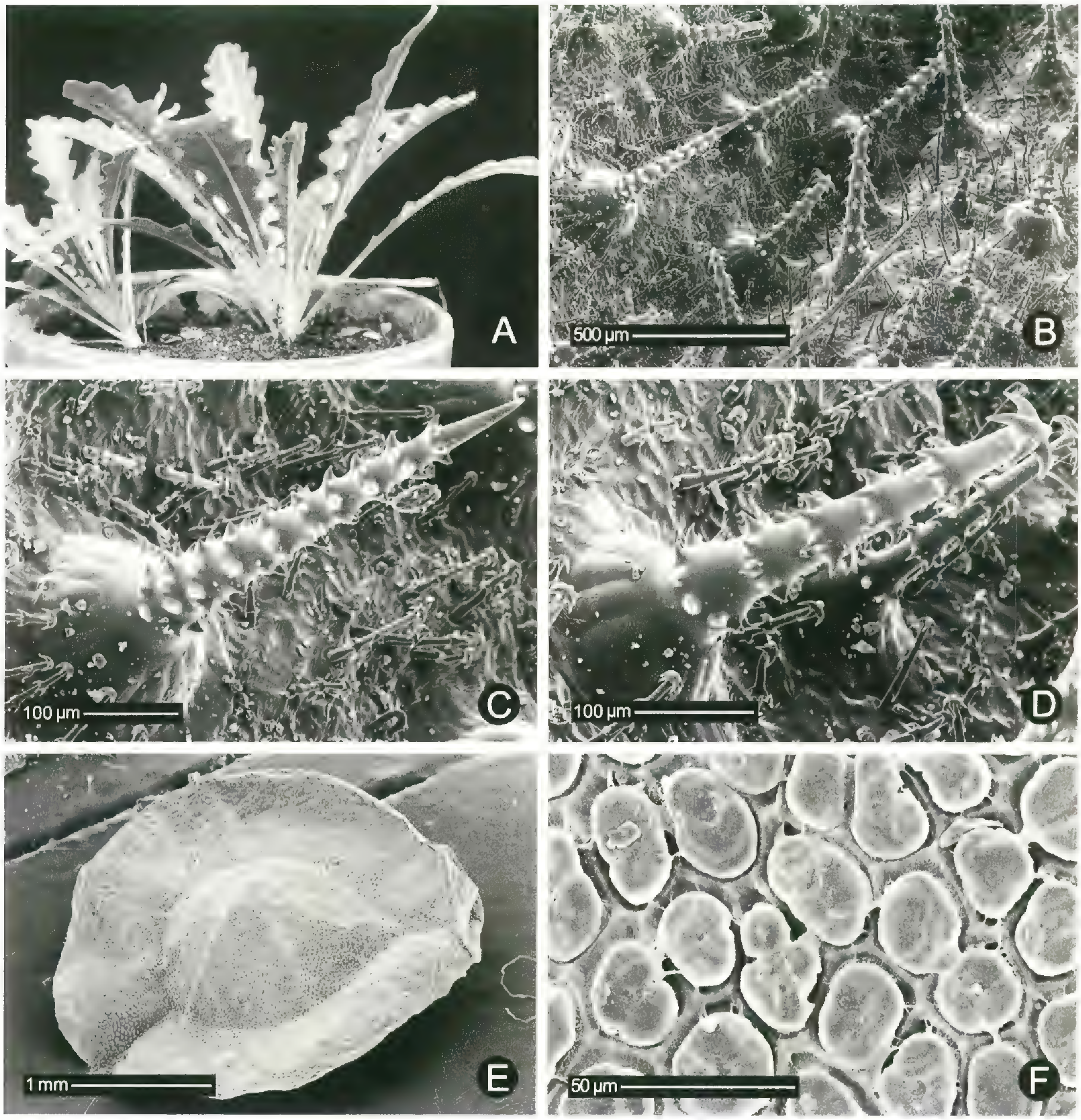


Figure 1. *Mentzelia albescens*. —A. Young plant showing dense rosette of lyrate leaves. B–D. Indumentum on abaxial ovary surface. —B. Overview, mixture of long glochidiate, scabrid, and short glochidiate trichomes. —C. Individual, long scabrid trichome. —D. Individual, long glochidiate trichome. E, F. Seed. —E. Entire seed, note the wing. —F. Detail of testa epidermis. From *Weigend et al. 6865*.

Distribution and habitat. *Mentzelia albescens* grows in dry, semi-desert situations, typically on sand or gravel soils, at elevations of 150–1000 m in Chile and 150–1600 m in Argentina and has also been reported from Argentina, from the province San Luis (Perez-Moreau & Crespo, 1988), and from the provinces La Pampa and Tucumán (Sleumer, 1955), in addition to the provinces listed below.

Additional specimens examined. ARGENTINA. **Catamarca:** Dpto. Capital, Río del Valle, *Venturi 6071* (MO). **Córdoba:** Córdoba, *Hieronymus 9888* (BR), *Lorentz 395* (GOET), *Lorentz 43* (GOET, M); Río Primero, *Hunziker 10500* (MO). **Mendoza:** 40 km NW of Mendoza, *Conrad 2662* (MO); Luján, Cacheute, *Balegno & Barkley 20MZ.282* (MO). **Neuquén:** Bajo del Marucho, *Schajovsky 60* (M); Lacar, Pampa de Alicura btw. Confluencia & Alicura, *Weigend et al. 6865* (B, BRCO, NY); Los Lagos, Cerro Bayo near Villa La Angostura, *Weigend et al. 6866* (B, BRCO, NY); Picunches, Quebrada Las Lajitas 3 km W of Las Lajas, *Weigend et al. 6912* (B, BRCO, NY). **Río Negro:** Near General Roca, Río Negro, *Fischer 61* (MO). **San Juan:** Iglesia, El Rodeo, *Cuezzo 1843* (WISC); Jachal, rd. to Mogne, *Kiesling 3008* (MO). CHILE. **IV Región, de Coquimbo:** Rivadavia, *Werdermann s.n.* (MO); Elquí nahe Vicuña, *Vogel 277* (WU); Río Elqui, 69 km from La Serena, 10 km E of La Vicuña, Quebrada Tchuni, *Rosas 1428* (M); riverbed near Tololo, *Bayer 530*

(M); Dpto. Ovalle, La Torca, near Tongoy, *Jiles 528* (M); close to Carén, *Jiles 4689* (M); Choapa, Choapa valley, 10 km S of Illapel, *Grau 314* (M). **Province unknown:** *Bridges s.n.* (E), *Gay s.n.* (B).

2. Mentzelia angurate Weigend, sp. nov. TYPE: Peru. Lima: Huarochirí, btw. Chosica & Matucana on Carretera Central, 1900 m, *M. Weigend, N. Dostert & K. Drießle 97/459* (holotype, USM!; isotypes, CPUN!, F!, M!). Figures 2, 3.

Haec species *Mentzeliae scabrae* affinis, sed ab ea ovario trichomatibus scabridis, flavescentis, patentis, usque ad 2 mm longis, instructo differt.

Subshrub 0.3–0.6 m tall, decumbent to ascending; stems 5–8 mm thick basally, with pale, strongly exfoliating bark, lignescent, young shoots densely covered with erect scabrid and glochidiate trichomes. Leaves subsessile or shortly petiolate (lower leaves with petioles 2–4 mm); lamina ovate, 20–30(–53) × 18–25(–30) mm, shallowly and irregularly lobed or just deeply serrate; base subcordate to subcuneate; apex acuminate. Inflorescence frondose, lax, widely branched (branching angle between paraclades of dichasia < 90°); internodes 2–4× as long as subtending bracts, bracts 15–20 × 10–15 mm; pedicels 0–1 mm. Flowers with calyx tube subglobose, 4 × 4 mm, densely covered with half-erect to erect, yellow, scabrid trichomes; calyx lobes narrowly ovate and long-acuminate, ca. 10 × 3 mm; petals widely obovate, 18–25 × 8–12 mm, apex apiculate, bright orange; stamens ca. 60, 10 outer ones markedly longer, ca. 13 mm, inner ones 5–6 mm, anthers 1 × 0.7 mm. Capsule globose, 6–8 × 6–8 mm, very densely covered with scabrid trichomes, these acroscopic to appressed, 1200–2000 µm long, pale yellow to beige.

Mentzelia angurate is excellently documented in herbaria, and the main reason why this taxon has been consistently overlooked is that it has usually been identified as *M. cordifolia* Dombey ex Urb. & Gilg (= *M. scabra* Kunth subsp. *grandiflora* (G. Don) Weigend). The descriptions by Urban and Gilg (1900) and Darlington (1934) both note the long trichomes on the ovary (see Fig. 3A, B) and thus agree with this identification; however, the type of specimens of *M. cordifolia* that I reviewed do not match this description. The extant *Dombey* specimens seen in G and P (cited in the protologue of *M. cordifolia*; Urban & Gilg, 1900) lack the long trichomes on the ovary and clearly belong to *M. scabra* subsp. *grandiflora* (see 7.5 below for further details); they were likely collected in the Lomas de Chancay, where *M. angurate* is not found. A distinct and new name is therefore required for the taxon. The characteristic very long, yellow trichomes on the ovaries make it readily distinguishable. The new species is more or less decumbent and is a smaller subshrub than either typical *M. scabra* or *M. sericea*.

Distribution, habitat, and etymology. The plants are found on steep scree slopes with open vegetation and also on road banks at elevations of 800–3000 m. Its peak flowering time is March to May, with sporadic flowering throughout the year, depending on the availability of moisture. It is largely restricted to the Peruvian departments of Lima and the extreme south of Ancash, and is replaced by *M. scabra* subsp. *chilensis* both north and south of this region. We know of a few populations from Huánuco that may have been dispersed by humans, since the central highway ("Carretera Central," a main gateway for people and goods for centuries) from Lima to Huánuco connects the province Huarochirí, where the species abounds, with the valley of Huánuco. This conclusion is supported by the fact that at least one other species that is otherwise restricted to the western slope of the Andes is found in this precise region and nowhere else in Andean valleys (*Nasa chenopodiifolia* (Desr.) Weigend). The specific epithet "anguraté" (or "anguaraté") represents the local name of *Mentzelia* in central Peru.

Paratypes. PERU. **Ancash:** Recuay, Barranca, rd. from Conococha to Pativilca, *M. Weigend, K. Weigend, T. Henning & Ch. Schneider 5549* (BSB, HUT, M, USM); Bolognesi, 3 km below Chiquián, *R. Ferreyra 7377* (USM); Bolognesi, 10 km SE of Chiquián, *K. Young & M. Eisenberg 925* (MO). **Huánuco:** Huánuco, *J. F. Macbride 3215* (G); Ambo, rd. from Huánuco to Cerro de Pasco, Km 27, 3 km from Ambo, *M. Weigend, K. Weigend, T. Henning & Ch. Schneider 5465* (BSB, HUT, M, USM); Huánuco, Cerros de Huánuco, *R. Ferreyra 1572* (USM). **Lima:** Canta, path to Canta, *R. Ferreyra 8718* (M, USM); near Canta, *R. Ferreyra 7248* (M, USM); ca. 2 km below Canta, *G. Yarupaitán 1674* (M, USM); Huarochiri, San Bartolomé, *N. Valencia 1416* (M, USM); Tambo de Viso, Km 83–84 on Carretera Central, *G. Yarupaitán 1675* (M, USM); Yaso, *G. Vilcapoma & M. Flores 1141* (M, MOL); Canta, *R. Ferreyra 7248* (M, MOL); Tornamesa, btw. Chosica & Surco, *R. Ferreyra 7030* (M, USM); Surco, Km 74 of Carretera Central, *R. Ferreyra 4079* (USM); just above (ENE) Surco, Km 72 on Carretera Central, *D. & V. Ugent 5293* (WIS); Puerta Huarimayo, *G. Vilcapoma s.n.* (M, MOL); Ayasuna, *M. Flores 180* (M, MOL); Río Chillón, Viscas, *F. W. Pennell 14472* (K, US, USM); Matucana, *R. Ferreyra 8286* (M, USM); Canyon of Río Rimac, 6 km above Matucana, *P. Hutchison & M. Ricardi 4987* (M, USM); betw. Chosica & Matucana on Carretera Central, *M. Weigend, N. Dostert & K. Drießle 97/459* (F, HUT, M, USM); Surco, Km 74 of Carretera Central, *R. Ferreyra 4979* (M, USM); Km 70 of Carretera Central, Huarochiri, *Ferreyra 3489* (USM), *R. Ferreyra & E. Cerrate 14887* (USM); Huarochirí, rd. Santa Eulalia to San Juan de Iris, *N. Smith & B. León 1362* (USM); San Bartolomé, *E. Asplund 10885* (G); Río Rimac, *Ball. s.n.* (K); Matucana, *J. F. Macbride & W. Featherstone 130* (G); 6 km ENE of Matucana, *D. & V. Ugent 5297* (K, USM, WIS); Km 69–70

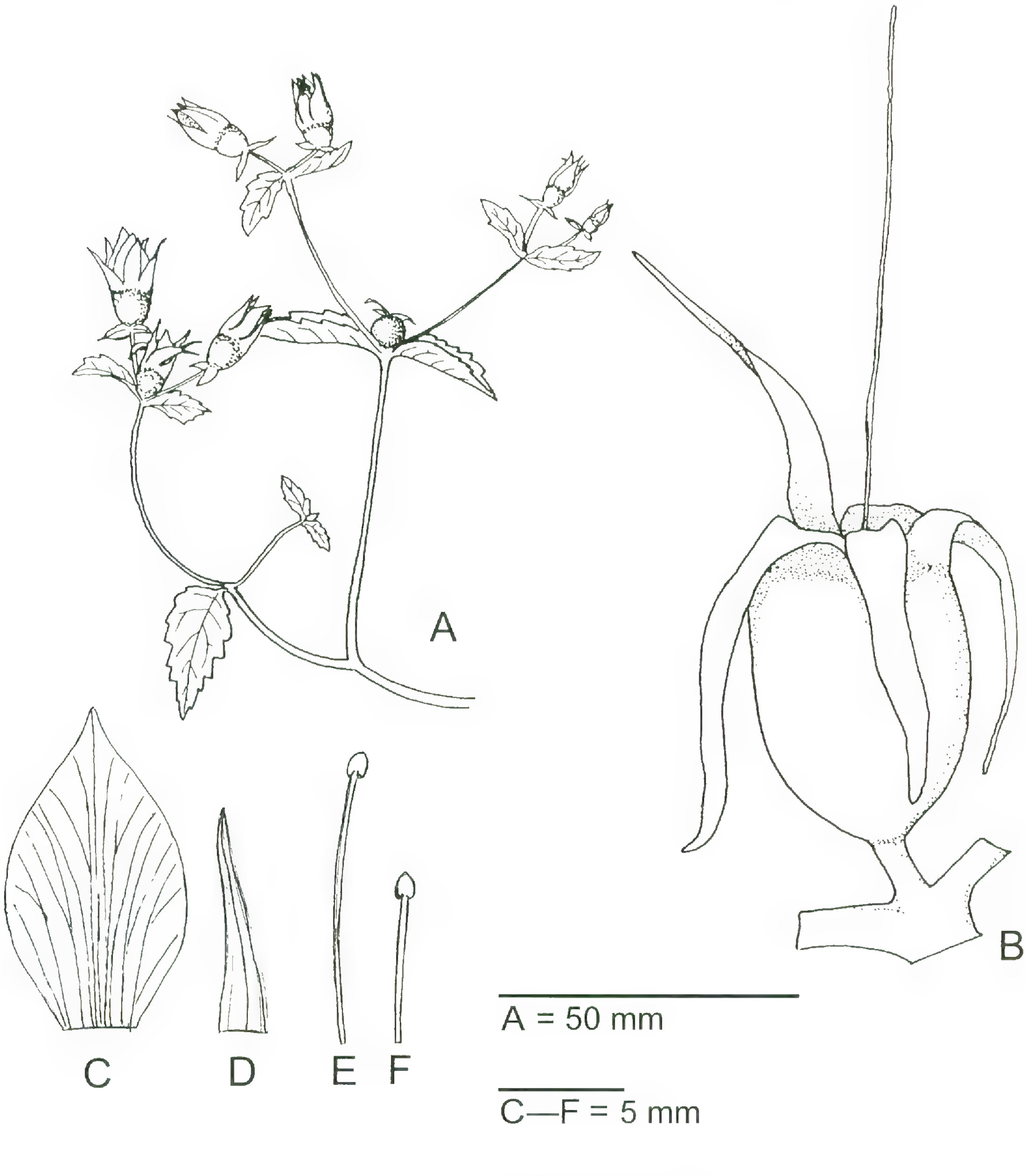


Figure 2. *Mentzelia angurate* Weigend. —A. Inflorescence. —B. Fruit. —C. Petal. —D. Sepal. —E. Outer stamen. —F. Inner stamen. Drawn from *Ugent & Ugent 5293.*

of Carretera Central, near bridge, *R. Ferreyra 2018* (USM); Tamboraque, Oroya railway, *Sandeman s.n.* (K); Km 70 of Carretera Central, *R. Ferreyra 14887* (M, USM); near Surco, Oroya, *L. Martinet 167* (P), *L. Martinet 939* (P); Matucana, *J. P. Hjerting & E. Petersen 1229* (USM); Matucana, *A. Weberbauer 89* (G); btw. Chosica & Matucana on Carretera Central, *M. Weigend, N. Dostert & K. Drießle 97/459* (F, HUT, M, USM); Chosica, *R. Ferreyra 6111* (USM); Km 56 on Carretera Central, *R. Ferreyra 8236* (M, USM), *R. Ferreyra 590* (USM); Canyon of Río Rimac, 6 km above Matucana, *P. Hutchison & M. Ricardi 4987* (K, USM); Surco, *P. Nuñez 1660* (M, MOL); Quebrada San Juan btw. Surco & Matucana, *R. Ferreyra 5430* (M, USM); S of Matucana along Carretera Central, *G. Sullivan et al. 835* (MO); Chaneay, Sayan, near the ruins, *H. Beltrán 2* (USM). **Province unknown:** *L. Martinet 74* (P), *L. Martinet s.n.* (P), *J. McLean s.n.* (K), *P. A. Savatier s.n.* (K).

3. Mentzelia aspera L., Sp. Pl. 1: 516. 1753. TYPE: tab. 174. in C. Plumier. Nov. Pl. Amer. 1703. Figure 4.

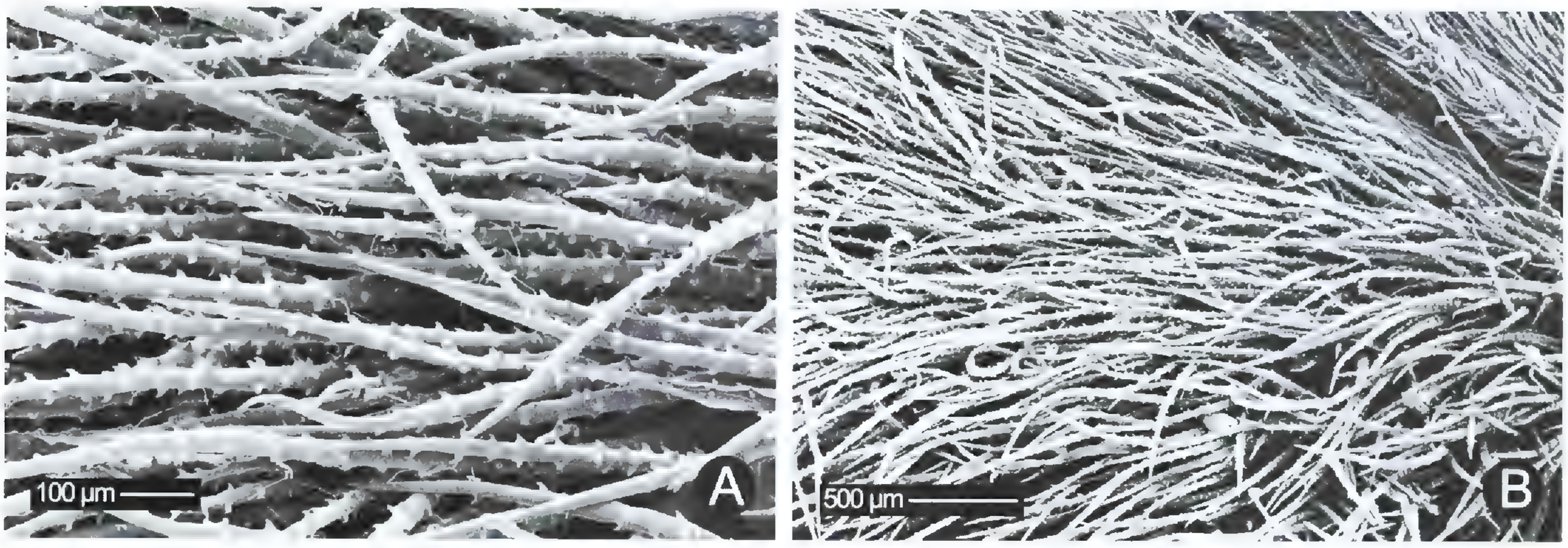


Figure 3. *Mentzelia angurate* Weigend, indumentum on ovary. —A. Overview, indument of scabrid trichomes of more or less equal length. —B. Close-up showing thickly overlapping layers of long, scabrid trichomes (epidermis not visible). From *Weigend et al. 5549.*

Mentzelia propinqua F. Aresch., Pl. Itin. Eugeniae: 133. 1910. TYPE: Ecuador. [Guayas:] Isla de Puná, *N. J. Andersson 124* (lectotype, designated by Weigend, 2000: 9, S!).

Mentzelia fragilis Huber, Bull. Herb. Boissier, ser. 2, 1: 314. 1901. TYPE: Brazil. Ceará: Quixada reservoir, btw. rocks, *J. Huber s.n.* (lectotype, designated here, G!; isotype, SP not seen).

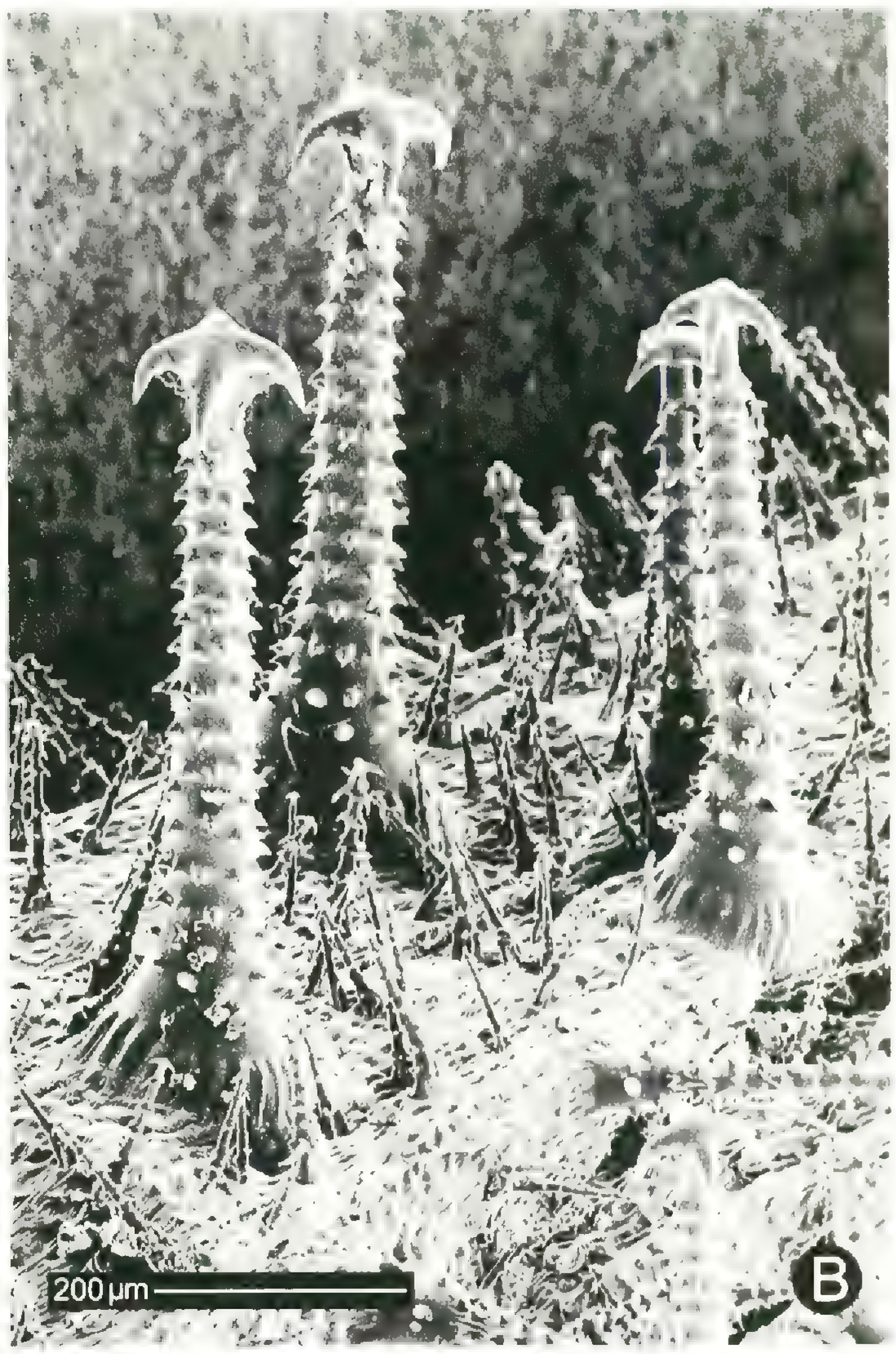
Mentzelia triloba Ruiz & Pav., Anales Inst. Bot. Cavanilles 16 (Fl. Peruv. 5): 423. 1958. TYPE: tab. 444, fig. a, in Ruiz & Pavón, 1958.

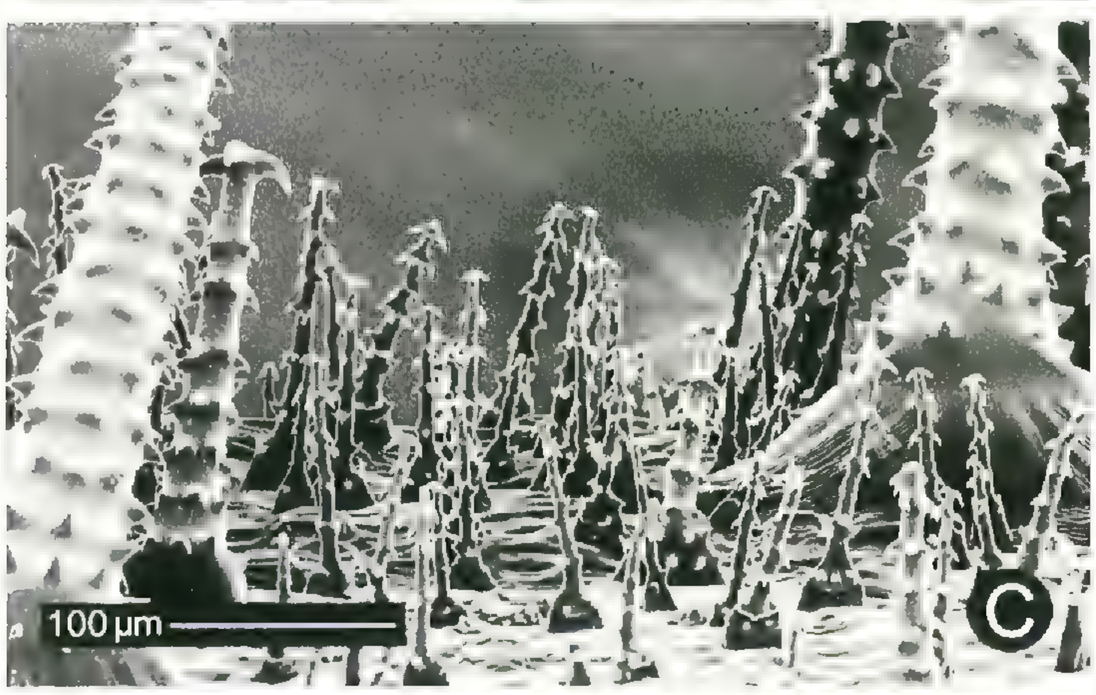
Erect, decumbent or straggling annual herb (0.1–) 0.3–1.5(–2) m tall; stem up to 6 mm thick, densely set with erect glochidiate trichomes 0.6–1 mm long and with scabrid, apically tapering trichomes 0.1–0.3 mm long; stem and branches slightly succulent and extremely brittle. Leaves spirally inserted, petioles 10–40(–80) mm; lamina ovate to triangular-ovate, 40–110(–160) × 20–80(–120) mm; base truncate to cuneate; apex acuminate; margin coarsely serrate, with 1 ovate to oblong lobe on each side, lobes 5–25 × 10–25 mm. Inflorescences as terminal dichasia or thyrsoids, widely branched (branching angle between paraclades of dichasia ca. 90°) with opposite, recaulescent (to the next flower), frondose bracts. Flowers with calyx tube shortly cylindrical, 4–8 × 1.5–2 mm, slightly narrowed at base, with dense cover of glochidiate hairs 50–700 µm long, calyx lobes narrowly ovate to linear, 5–8 × 2 mm; petals widely ovate to subcircular, 5–15 × 2.5–7 mm, base narrowed, apex acute, golden yellow to pale orange; stamens 20 to 30, 10 outer ones with filiform or petaloid filaments, all filaments ca. 5 mm, anthers ca. 1 × 1 mm, ovate to oblong, yellow; style filiform, ca. 5 mm, with 3 decurrent stigmas. Capsule initially erect, then deflexed, narrowly cylindrical and only slightly narrowed at base, 9–25 × 2.5–3.5 mm, covered with glochidiate trichomes, longer ones 500–600 × 100 µm, with 12 to 16 bands of short hooks, short ones rather uniformly 100–130 × 20 µm thick with 5 to 6 bands of short hooks, seeds 6 to 9, irregularly oblong, 2.5–3.5 × 1.5 mm, yellowish gray.

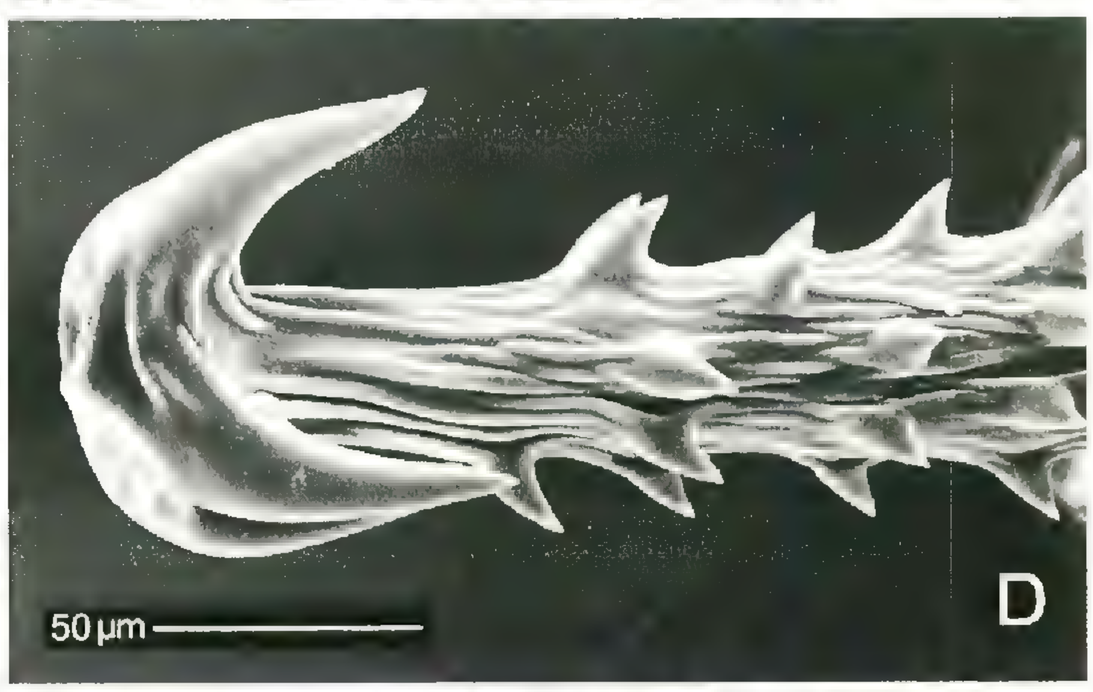
Mentzelia aspera is a widespread and variable species. In semi-desert habitats, the plants may flower when barely reaching 10 cm in height, producing less than five flowers per plant, whereas other individuals have very large leaves (16 × 12 cm) and reach a height of 1.5–2 m (Bolivia, *Fuentes 1519*). The segregate *M. fragilis* proposed for the Brazilian specimen (*J. Huber s.n.*) is not accepted. The Brazilian material studied by this author agrees perfectly with the Andean and Central American specimens. Two possible type specimens are extant, and lectotypification for *M. fragilis* is required. It is here lectotypified on the specimen at G, since the SP specimen was not seen. The specimens from the Galápagos Islands tend to have the smallest leaves of any specimens seen for this revision, and therefore several infraspecific segragates have been proposed for the Galápagos forms (Urban & Gilg, 1900). They are not accepted here because they grade into typical *M. aspera* in other specimens from the Galápagos Islands.

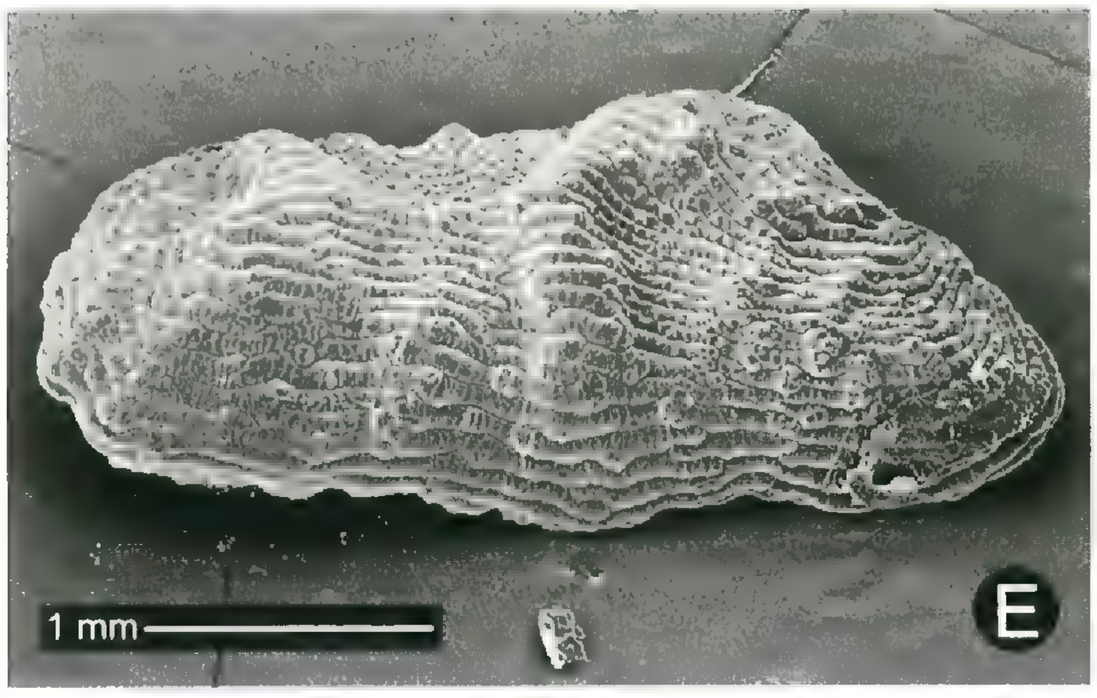
Distribution and habitat. Most collections of the species are from situations where it grows as a weed, but it is also an important component of tropical dry forest and cactus scrub in Peru. It grows from sea level to elevations of 1500(–2000) m, and flowers and fruits whenever sufficient moisture is available.

Additional specimens examined. ARGENTINA: **Salta:** Oran, Misión Zenta, *Sleumer s.n.* (B); Salta, Capital, Cerro San Bernardo, *Novara 2417* (MO), *Novara 2351* (MO). **Tucumán:** Burruyacu, *Venturi 10322* (MO). BOLIVIA. **Cochabamba:** Near Cochabamba, *Bang 1005* (M), *Bang 748* (M). **La Paz:** Bautista Saavedra, Charazani, above









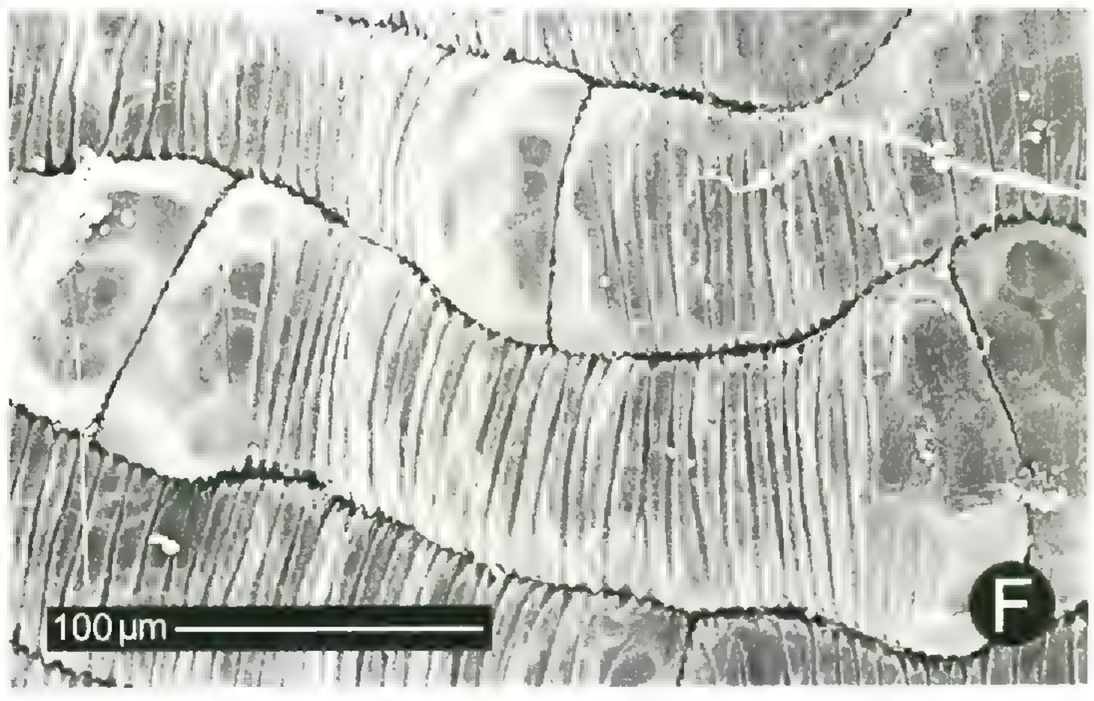


Figure 1. *Mentzelia aspera*. —A. Androecium of anthetic flower of *M. aspera*, showing slightly longer and widened antesepalous filaments (sf) and the shorter, antepetalous, filiform filaments (pf; *Weigend et al. 5733*). B–D. Indumentum on ovary (*Weigend et al. 5733*). —B. Mixed indumentum with short and long glochidiate trichomes. —C. Short glochidiate trichomes between bases of long glochidiate trichomes. —D. Apex of long, glochidiate trichome. E, F. Seeds (*Rodriguez & Campos 1849*). —E. Overview. —F. Detail of testa epidermis.

Carijana, *Gutte & Birger Herzog 595* (LPB, LP, MO). **Nor Yungas:** Millugnaya, *Buchtien 4357* (NY); Canamina, *Troll 1640* (B, M). **Santa Cruz:** Cordillera, turnoff 3 km N of bridge over Río Seco, *Nee 49060* (BSB, NY), *Nee & Bohs 49448* (BSB, NY); Nuflo de Chávez, Estancia San Miguelito, 200 km NE of Santa Cruz city, *Fuentes 1519* (MO). **Sud Yungas:** Chulumani, 25 km toward Asunta, *Beck 12051* (M, UMSA). BRAZIL. **Bahia:** Bahía, Serra do Ramalho, 10 km S of town, *Jardim et. al. 3456* (NY); Mpio de Bom Jesus da Lapa, 16 km on rd. to Ibotirana, *Carvalho et al. 3948* (CEPEC, M); 3–4 km N of Mpio de Livramento de Nossa Senhora on rd. to Río de Contas, *Jardim et al. 2033* (BSB, CEPEC, M, NY); Canabrava, Mun. Oliveira dos Brejinhos, *Hatschbach et al. 67778* (MBM); Serra do Brejo Grande, Serra Geral, Mun. Boquira, *Hatschbach et al. 67606* (MBM); Bom Jesus da Lapa, near Bahia, *Hatschbach et al. 55192* (MBM); 10 km N de Guanambi, *Hatschbach et al. 61904* (MBM). **Ceará:** Grangeiro, Taboleiras, *von Luetzelburg 24493* (M); La Muralha, *von Luetzelburg 26474* (M). **Mato Grosso de Sul:** Estr. do Gaturama, Mpio. Corumbá, *Hatschbach 29495* (MBM). **Minas Gerais:** 15 km N of Montâlvania, *W. R. Anderson et al. 37180* (WIS); Serra do Espinhaço, Mun. Monte Azul, *Hatschbach et al. 61897* (MBM). **Paraiba:** Paraiba, *de Moraes 2113* (B, MO). CAPE VERDE ISLANDS. **Santo Antão:** N coast, 1 km E of Cruzinha and Lacacão, *Kilian & Leyens 2902* (B). **San Vicente:** Monte Verde, *Kilian 750* (B), *Royl 844* (B). COLOMBIA. **Antioquia:** 2 km N of Antioquia, *Gutiérrez & Barkley 75C089* (COL); Mpio de Antioquia, rd. Santa Fé to Antioquia–Anzá, *Zarucchi et al. 5493* (MO). **Atlántico:** Cerca de la playa Miramar, *Dugand 4412* (COL); Usiacuri, *Barkley 19043* (COL). **Cauca:** Mercaderes, Mojarras, Granja de la Universidad de Nariño, *de Benavides 10605* (PSO). **Cundinamarca:** Cordillera Oriental, W slope, near Puente San Antonio de Tena, *Cuatrecasas 8264* (COL). **Magdalena:** Río Azucarbuena, El Callao, Hac. Santa Marta, *Cuatrecasas & Romero-Castañeda 24932* (COL); Fonseca, *Haught 4395* (COL); Cerro San Fernando, *Schnetter & Schnetter 134* (COL); Gaira, Mpio de Santa Marta, *Romero-Castañeda 10471* (MO); Parque Tairona, Canaveral, trail to Arrecifes, *Plowman & Davis 3715* (COL); Santa Marta, *Smith* 477 (BR, MO); rd. La Gran Vía to San Pedro, *Romero-Castañeda 10640* (MO); Mpio de Fundación, Corregimiento Santa Rosa, *Romero-Castañeda 11062* (MO). **Nariño:** Chachagüi, puente del Juanambú, carretera Panamericana, *de Benavides 3631* (PSO); Taminango, Km 68 Panamericana Pasto-Popayán, *de Benavides* 5412 (PSO). **Santander:** En el Cañon de Chicamocha y el Valle de Rupala, *Saravia 845* (COL). **Sucre:** Trail from Colosó to Reserva de Primatas, *Gentry et al. 34758* (COL); Mpio Coloso, Bosque Protector of Estación de Primates Finca El Sereno, *Cuadros-V. & Gentry 4656* (MO). **Valle del Cauca:** Btw. Loboguerrero & Yumbo, *Saravia 1655* (COL). **Province unknown:** La Picota, *Schwabe s.n.* (B). ECUADOR. **Esmeraldas:** Atacames Beach, *Webster 22801* (MO); Hac. Guayas, 20 km S of Esmeraldas, *Sparre 15533* (S); 1 km S of city of Esmeraldas, *Hudson 722* (MO). **Guayas:** Isla de Puna, *N. J. Andersson* 125 (S); Chanduy, *Spruce 6463* (E); 3 km N of Julio Moreno (Simón Bolívar), *Harling & L. Andersson 25017* (GB, M); 1 km NW of Chanduy toward Atahualpa, *Holm-Nielsen et al. 2137* (GB); Guayaquil, *Asplund 16649* (S); Playas, *Asplund 5036* (S); Capeira, 22 km on rd. from Guayaquil to Daule, *Gentry & Dodson 54785* (MO); Capeira, 21 km on rd. from Guayaquil to Daule, *Dodson & Gentry 12470* (MO); Bosque Protector Cerro Blanco, *Tipaz et al. 817* (MO); Cerros de Masvale halfway btw. Boliche (Pedro J. Montero) & Jesus María, *Harling & Andersson 19261* (GB); Guayaquil, golf club, *Harling & L. Andersson 8815* (GB); 3 km N of Julio Moreno (Simón Bolívar), *Harling & L. Andersson 25017* (GB); Guayaquil, railway to Salinas, *Asplund 7702* (S); 20 km S of Manglaralto, near Palmar, *Holm-Nielsen et al. 2486* (S); Guayaquil, Universidad de Guayaquil, *Cornejo & Bonifaz 5648* (GUAY, M). **Islas Galápagos:** Isla Baltra, N part of the island, *Eliasson & Eliasson 1987* (GB); Santa Cruz, Darwin Research Station, *Bentley 206* (QCA); Champion Island, *Eisendrath s.n.* (MO); Wreck Bay, *Stewart 2086* (MO); Indefatigable Island, Academy Bay, *Howell 9055* (B), *Stewart 2087* (B); Albemarle Island, Iguana Cove, *Stewart 2083* (B). **Loja:** San Pedro de la Bendita to El Cisne, Km 8, *Øllgard et al. 90703* (QCA). **Manabí:** Portoviejo–Jipijapa rd., 1 km S of La Pila, *Harling & L. Andersson 24827* (GB, M); 5 km N of Jipijapa, *Haught 3397* (S); Manta, *Asplund 15945* (S); Puerto Lopez, PN Machalilla, Isla Salango, *Cerón 18758* (BSB, MO); Isla de la Plata, path to Faro, *Cerón 18727* (BSB, MO). **Province unknown:** s. loc., *André* 89 (K). PERU. **Amazonas:** Bagua, trail from La Peca to El Arenal, *Dostert 98/79* (CPUN, F, M, USM); Bagua, btw. La Peca & Bagua Chica, *Barbour 4267* (MO); Bagua, Cumba, btw. Tactago & Chiñuña-Malleta, *Campos et al. 6108* (MO); Bongará, Jasán, Aserradero, *Vásquez & Rojas 26486* (MO). **Ancash:** Moro, rd. from Moro to Pamparomas, *Weigend et al. 8121* (B, HUT, USM). **Apurímac:** Abancay, rd. from Abancay to Andahuaylas, *Weigend & Weigend 2000/312* (NY, USM). **Cajamarca:** Chota, Llama, 28 km from Llama on rd. to Chiclayo, *Weigend & Dostert 98/166* (CPUN, F, M, USM); Chota, rd. Olmos to Jaén, Bagua, E slope of Abra Porculla, *Weigend et al. 8609* (B, HUT, USM); Chota, btw. Chota & Lajas, *Weigend et al. 7524* (B, HUT, MO, USM); Santa Cruz, Hac. Espinal, ca. 70 km E of Panamericana Norte, *Ferreyra 12392* (USM); Jaén, Jaén to San Ignacio, 45 km from Jaén, *Weigend et al. 98/459* (CPUN, F, M, USM); 9–16 km E of Pucará on rd. to Bagua, *Gentry et al. 22729* (USM); Huehuaya to Peña Blanca (Tamborapa–Tabaconas), *Leiva et al. 1239* (HAO, M); San Ignacio, Distr. Coipa, Tamborapa, left bank of Río Tabaconas, *Campos & Díaz 3001* (BSB, MO, USM); San Ignacio, Distr. Chirinos, Cenezal, *Campos & Díaz 2189* (BSB, MO); San Ignacio, Huarango, btw. San Martín & La Mushca, *Campos et al. 6222* (MO); San Ignacio, Distr. Chirinos, Caserío Las Juntas, banks of Río Tamborapa, *Rodriguez & Campos 1849* (B, HUT, MO); San Ignacio, Distr. San Ignacio, btw. Puerto La Naranja & Puerto Huaquillo, *Rodriguez & Campos 1857* (B, HUT, MO). **Cuzco:** Convención, Quillabamba, *Vargas 7107* (CUZ); Chawares, *Vargas 20784* (BSB, CUZ); La Convención, Huayopata, San Luis, *Galiano et al. 4915* (BSB, MO); Convención, Potrero, *Vargas 2144* (MO). **Junín:** Satipo, rd. from San Ramón to Satipo, 2 km from Santa Ana, *Weigend et al. 5733* (BSB, HUT, M, USM). **La Libertad:** Trujillo, Santa Rosa near Trujillo, *Sagástegui & Araujo 7783* (HUT, NY); Laguna San Bartolo, near Ascope, *Müller & Krebs 12129* (USM); Santa Clara, Valle Chicama, Trujillo, *López s.n.* (HUT 0799); Santa Rosa (alrededores de Trujillo), *Sagástegui & Ramirez 13063* (HUT); San Antonio, Ascope, *López & Sagástegui s.n.* (HUT 2530); Ascope, Mocan, *Sagástegui 14905* (MO). **Lambayeque:** Lambayeque, Sapote, Motupe, *Llatas-Quiroz 3133* (MO); Ferreñafe, old rd. Tumán-Patapo to Batan Grande (destroyed in the 1982 El Niño, now replaced by direct route Ferreñafe–Batan Grande), *Weigend et al. 8516* (B, HUT, USM); Ferreñafe, rd. from Batan Grande to Incahuasi resp. Ullurpampa (syn. Uyurpampa), *Weigend et al. 8523* (B, HUT, USM). **Lima:** Huarochiri, Santa Rosa de Chontay, *Cano 1139* (M, USM). **Piura:** Chulucanas, rd. Huancabamba to Chulucanas, btw. Malacasí & Buenos Aires, *Weigend & Dostert 98/287* (CPUN, F, M, USM); rd. Chulucanas to Frías, near Papelillo, *Weigend*

& *Dostert 98/178* (CPUN, F, M, USM); near Huancabamba, *Acleto 469* (USM); Tablazo River, *Haught 217* (COL); Chulucanas, Cerro Vicus, 52 km from the rd. to Piura, Morropon, *Sánchez & Guevara 6196* (CPUN, M). **San Martín:** Granja Bellavista, *Chrostowski 70-280* (WIS); San Martín, Vaca Pozo, Río Cumbaza, SE of Tarapoto, *Woytkowski 35487* (USM); along rd. Juanjuí to Tarapoto, 35 km S of Tarapoto, *Croat 58081* (USM); Tarapoto, *Spruce 4922* (BR); Fundo San Isidro (Tarapoto), *Sagástegui 8324* (HUT); Chazuta, Río Huallaga, *Klug 4030* (MO); San Martín, Tarapoto, Vaca Pozo, *Woytkowski 35181* (M). **Tumbes:** La Peña 16 km SE of Tumbes, *Ferreyra 5987* (USM). VENEZUELA. **Aragua:** Lago de Valencia, Isla Bruja, *Vareschi & Gessner 1937* (M); btw. Maracay & Mariana, *Alston 5688* (WIS). **Carabobo:** Saman Mocho, experimental fields of the Facultad de Agronomía de al UCV, *van Rooden & Akkermans 131* (WIS). **Distrito Federal:** Llanos near Chaguaramas, *Schwabe s.n.* (B); Cordillera de la Costa, old rd. from Caracas to La Guaira, *Oberwinkler 14528* (M); Libertador, valley of Río Chichiriviche, 0.5 km S of Chichiriviche, *Steyermark & Carreño 112726* (MO, NY); near Colonía Tovar, *Fendler 453* (MO). **Lara:** Quebrada La Mata, 1 km N of Cabadure, 80 km SE of Barquisimiento, *Mejier & Durand 1* (MO, NY). **Maracay:** s. loc., *Vogel 1273 a/b* (M). **Mérida:** Ejido, *Weigend 3613* (M); Mérida, *Schwabe s.n.* (B). **Miranda:** Parque Nacional El Ávila, NNE of Caucagüita, *Steyermark et al. 113993* (MO). **Zulia:** Miranda, btw. El Mecocal & El Consejo, 16 km SE of El Mecocal, *Bunting 5997* (NY); along rd. (km 13, bridge over lake) to Los Crespos, *Bunting et al. 11818* (NY); Bolivar, rd. Lara–Zulia to El Zamoro, on roadside, *Bunting 8294* (NY).

4. **Mentzelia bartonioides** (C. Presl) Urb. & Gilg in Engl. & Prantl, Nat. Pflanzenfam. 3(6a): 110. 1894. Basionym: *Acrolasia bartonioides* C. Presl, Reliq. Haenk. 2: 39. 1835. TYPE: Tab. 55, in C. Presl, 1835. Figure 5.

Mentzelia andina Urb. & Gilg, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 76(1): 31. 1900, synonymized under *M. pinnatifida* by Urban & Gilg 1900: 356. TYPE: Chile. [IV Región, de Coquimbo:] Coquimbo, Cordillera de las Patas, *C. Gay 662* (lectotype, designated here, P!).

Mentzelia haenkii A. Gray, Pl. Wright. 1: 74. 1852, nom. inval., in nota.

Mentzelia pinnatifida (Phil.) Urb. & Gilg, Nova Acad. Acta Caes. Leop. Carol. German. Nat. Cur. 76(1): 356. 1900. Syn. nov. Basionym: *Acrolasia pinnatifida* Phil., Anales Univ. Chile 85: 6. 1893 [1894]. TYPE: Chile. [III Región, de Atacama:] Atacama, Quebrada del Molle, Bandurrias, *W. Geisse s.n.* (holotype, SGO not seen).

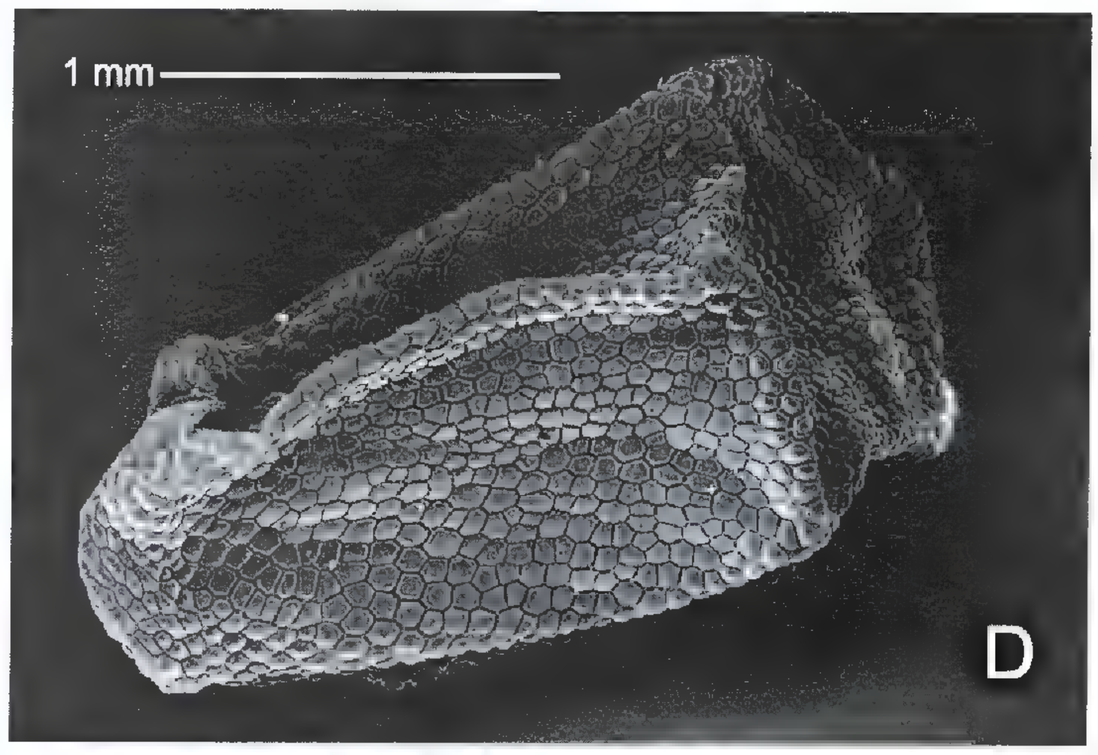
Mentzelia pinnatifida (Phil.) Urb. & Gilg var. *uniseriata* Hauman, Anales Soc. Ci. Argent. 86: 290. 1918. TYPE: Argentina. Mendoza: valley of Río Plumo, 2900 m, *L. L. Haumann 231* (lectotype, designated by Sleumer, 1955: 416, BA 17131 not seen; isotypes, BR!, G not seen).

Mentzelia solierii (Gay) Urb. & Gilg in Engl. & Prantl, Nat. Pflanzenfam. 3(6a): 110. 1894. Basionym: *Acrolasia solierii* Gay, Fl. Chil. 2: 430. 1846. TYPE: Chile. [III Región, de Atacama:] Copiapó, on dry hills near Copiapó, 1839, *C. Gay s.n.* (type, P!).

Annual herb 0.05–0.3 m tall, stiffly erect; stems basally 1–5 mm thick, shoot unbranched (in small individuals) to widely branched, with a single, little-branched taproot; stem off-white, densely covered with thin glochidiate trichomes. Leaves subsessile with long-tapering base, basal leaves few (in small individuals) or numerous in dense, short-lived rosette, upper ones alternate; lamina narrowly obovate, 25–100 × 5–20 mm, sinuate-dentate to pinnatifid with lobules widely spaced; base long-tapering; apex rounded to acuminate. Inflorescence bracteose, few- to many-flowered, strongly basitonic, initially dense, later lax (branching angle between paraclades of dichasia < 90°); proximal internodes 1–3× as long as subtending bracts, distal internodes much shorter than subtending bracts; bracts ovate, 5–25 × 3–15 mm; flowers subsessile to sessile. Flowers with calyx tube conical, 5 × 3 mm, densely covered with erect, thin, glochidiate trichomes 0.1–0.6 mm long; calyx lobes 1–2 × 1–1.5 mm, ovate-acuminate; petals widely ovate, 2.5–5 × 2–4 mm, apex acuminate to apiculate, pale to golden yellow; stamens 10 to 30, filaments 1.5–2.5 mm, filaments of antesepalous stamens sometimes with distinctly widened base, anthers ca. 0.5 × 0.3 mm; style filiform, 1.5–2 mm, with 3 decurrent stigmas. Capsule sessile, cylindrical, 5–10 × 3–5 mm, opening with 3 apical valves; seeds 15 to 25, ca. 1.5 × 1.5 mm, very irregularly angular, greyish brown.

The protologue of *Mentzelia andina* cites both *Gay 662* (B, P) and *Philippi s.n.* (B) as types, and lectotypification is therefore required. Because the Berlin specimens are lost (B† photo at F 10132), the *Gay* collection is selected here as the only extant type specimen. Another synonym of *M. bartonioides* is *M. citrina* Urb. & Gilg, which was synonymized by Sleumer (1955).

There has been a trend toward recognizing fewer and fewer species in this group since the recognition of four distinct taxa by Urban and Gilg (1900). In the most recent studies (Sleumer, 1955; Pérez-Moreau & Crespo, 1988), only two taxa (*Mentzelia pinnatifida* and *M. bartonioides*) were recognized, supposedly differing in the density of the trichome cover of the stem base and the expansion of the inflorescence branches, both of which are ontogenetically variable. This is illustrated by a set of collections from one locality at varying elevations in Coquimbo (*Squeo 88152, 88105, 88005, 88024*), which span the entire range of characters given for the original four species with regard to fruit size, indument, and inflorescence structure. Neither Sleumer (1955) nor Darlington (1934) were able to clearly separate the taxa morphologically or geographically, and my own observations also fail to reveal any differentiation;



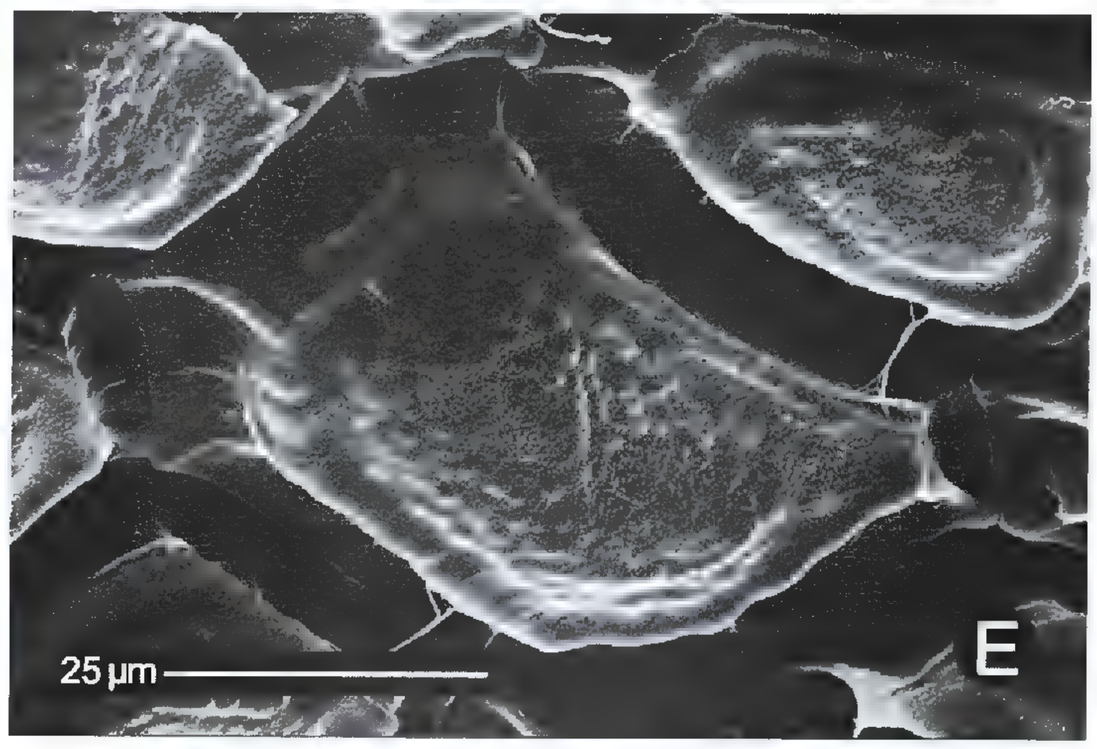


Figure 5. *Mentzelia bartonioides*. A–C. Habit. —A. Small, sparsely ramified plants (*Squeo 88005*). —B. Moderately ramified specimens (*Muñoz 2746*). —C. Well-developed individual (*Wagenknecht 18585*). D, E. Seeds (*Kiesling 8082*). —D. Highly irregularly shaped, sharply angled seeds. —E. Detail of testa epidermis.

thus, all South American taxa are reduced to a single name here.

Distribution and habitat. The species occurs in semi-desert vegetation and is a short-lived annual found at elevations of (1000–)2000–3500 m. The species is also reported from Mendoza, Argentina (Sleumer, 1955).

Additional specimens examined. ARGENTINA. **San Juan:** Calingasta, rd. from Río Blanco to Pachón, Las Juntas, *Ruthsatz 2650* (Hb. Ruthsatz–Universität Trier, Fachbereich VI Geobotanik), *Kiesl 88901* (Hb. Ruthsatz–Universität Trier, Fachbereich VI Geobotanik); campground, *Ruthsatz 8870* (Hb. Ruthsatz–Universität Trier, Fachbereich VI Geobotanik, M); Calingasta, Río Blanco (rd. to El Pachón), *Kiesling 8082* (MO, SI); Cordillera de Espinazito, Las Manantiales, *Stelzner* in *Kurtz 9755* (B photo at F 10135). CHILE. **III Región, de Atacama:** 11 km S of Inca de Oro, *Muñoz et al. 2746* (MO, SGO). **IV Región, de Coquimbo:** Quebrada El Pangue N of Chachiguaz, *Rosas 1478* (M); Cordillera de Doña Ana, *Squeo 88152* (MO, ULS), *Squeo 88105* (MO, ULS), *Squeo 88005* (MO, ULS), *Squeo 88024* (MO, ULS); Elqui, Los Lechuzos, 19 km E of Rivadavia, rd. to Laguna Dam, *Wagenknecht 18585* (MO, UC).

5. Mentzelia heterosepala Weigend & E. Rodr., Arnaldoa 5: 52, figs. 1, 2. 1998. TYPE: Peru. Amazonas: Bagua, rd. from Bagua Chica to Imauta, El Muyo, 1100 m, *E. Rodriguez R. 1298a* (holotype, M!; isotypes, F!, HUT!, USM!). Figure 6.

Shrub 1.5–2 m tall; stems up to 5 cm thick basally, ligneous, bark dark grey, exfoliating late or not at all, if exfoliating then in narrow, dark brown strips, without lenticels, young shoots very densely covered with very short glochidiate trichomes (< 0.5 mm long) and scabrid trichomes (ca. 0.5 mm long). Petioles 5–10 mm; lamina narrowly ovate, 50–80 × 20–25 mm, margin subentire to shallowly and remotely serrate; base cuneate; apex acuminate. Inflorescences frondose, often few-flowered, if many-flowered then with short paraclades barely overtopping the primary flower; bracts 15–25 × 5–8 mm, pedicels ca. 1 mm. Flowers with conical calyx tube ca. 10 × 3–4 mm, densely covered with very short glochidiate and scabrid trichomes, outer calyx lobes 18–20 × 8–10 mm, inner calyx lobes 16–18 × 5 mm; petals widely ovate, ca. 35 × 20 mm, base narrowed, apex acuminate; stamens 90 to 110, the 15 to 20 outer filaments ca. 25 mm long and erect from a spreading base, inner stamens 15 mm long, anthers ca. 1 × 0.75 mm; style filiform, ca. 30 mm, with 3 decurrent stigmas. Capsule clavate, ca. 10 × 4 mm, covered very densely with erect glochidiate and slightly acroscopic scabrid trichomes 100–200 µm long, scabrid trichomes longer than glochidiate trichomes, with a smooth apex, glochidiate trichomes with distinct 4- to 5-hooked apex and 3 to 4 bands of hooks; seeds 1 to 3, ca. 3.5 × 1.5–2 mm, greyish brown.

Mentzelia heterosepala is the only species of this genus in South America with clearly delimited, subcorymbose inflorescences and subentire leaves (Fig. 6A); it also has the most pronounced shrubby habit and the thickest branches of any seen South American *Mentzelia* species. With these characters, *M. heterosepala* resembles tree-like *M. arborescens* Urb. & Gilg from southern Mexico more than its South American congeners.

Distribution and habitat. Mentzelia heterosepala was recently collected (1996) for the first time in a single locality on the Río Marañón in Peru, but has now been reported from the Marañón Valley in four different departments and appears to be both widespread and locally abundant (in places dominating the vegetation of scree slopes). It is found at elevations of 900–1600 m, is restricted to steep scree slopes and rock slides, and is found very rarely on rocky outcrops.

Additional specimens examined. PERU. **Amazonas:** Chachapoyás, rd. Balsas to Leimebamba, *Weigend et al. 97/390* (F, M, USM); rd. Celendín to Chachapoyás, 8–10 km from Puente Chocanto, *Smith & Cabanillas 7053* (USM); rd. Balsas to Bolívar, before Longotea, *Weigend et al. 2000/804* (BSB, F, HUT, M, USM). **Ancash:** Pallasca, San José, near Conchucos, *Cano et al. 15444* (BSB, USM); Síhuas, rd. Síhuas to Huancaspata, *Sagástegui et al. 17426* (BSB, HAO, HUT); ascending the Río Rupac valley to Síhuas from Río Marañón, *Weigend & Schwarzer 8027* (BSB, HUT, USM), *Cano et al. 15846* (BSB, USM). **Cajamarca:** Celendin, rd. Celendin to Balsas, *Weigend et al. 97/388* (F, M, USM). **La Libertad:** Pataz, 5 km on rd. Chagual to Chugay (Huamachuco), W of Río Marañón, *Weigend & Schwarzer 7927* (BSB, HUT, USM); ca. 4 km on rd. Chagual to Tayabamba, E of Río Marañón, *Weigend & Schwarzer 7923* (BSB, HUT, USM); Pías, Quebrada El Oso, *Alayo 83* (HAO); btw. Chagual & Pataz, *Sagástegui et al. 17244* (BSB, HAO, HUT); btw. Chagual & Pataz, *Sagástegui et al. 17244* (BSB, HAO, HUT); Chagual, above Río Marañón, *Rodríguez 2543A* (HUT); Bolívar, btw. Pusac & Huanabamba, *Mostacero et al. 3608* (HUT).

6. Mentzelia parvifolia Urb. & Gilg ex Kurtz, Revista Mus. La Plata, Secc. Bot. 5: 289. 1893. TYPE: Argentina. Córdoba: Córdoba, Capitál, *F. Kurtz 614* (lectotype, designated here, CORD not seen). Figures 7, 8.

Mentzelia parvifolia Urb. & Gilg ex Kurtz var. *transiens* Sleumer, Bot. Jahrb. Syst. 76: 422. 1955. Syn. nov. TYPE: Argentina. Catamarca: Santa Maria, margin of Campo Arenal near Punta de Balastro, 2400 m, 3 Feb.–Mar. 1952, *H. O. Sleumer 2663* (holotype, LIL not seen; isotypes, S not seen, US [2]!, W!).

Acrolasia elata Phil., Anales Univ. Chile 85: 5. 1893 [1894]. TYPE: Argentina. Mendoza: rd. to Mendoza, *Goldsack s.n.* (holotype, SGO not seen).

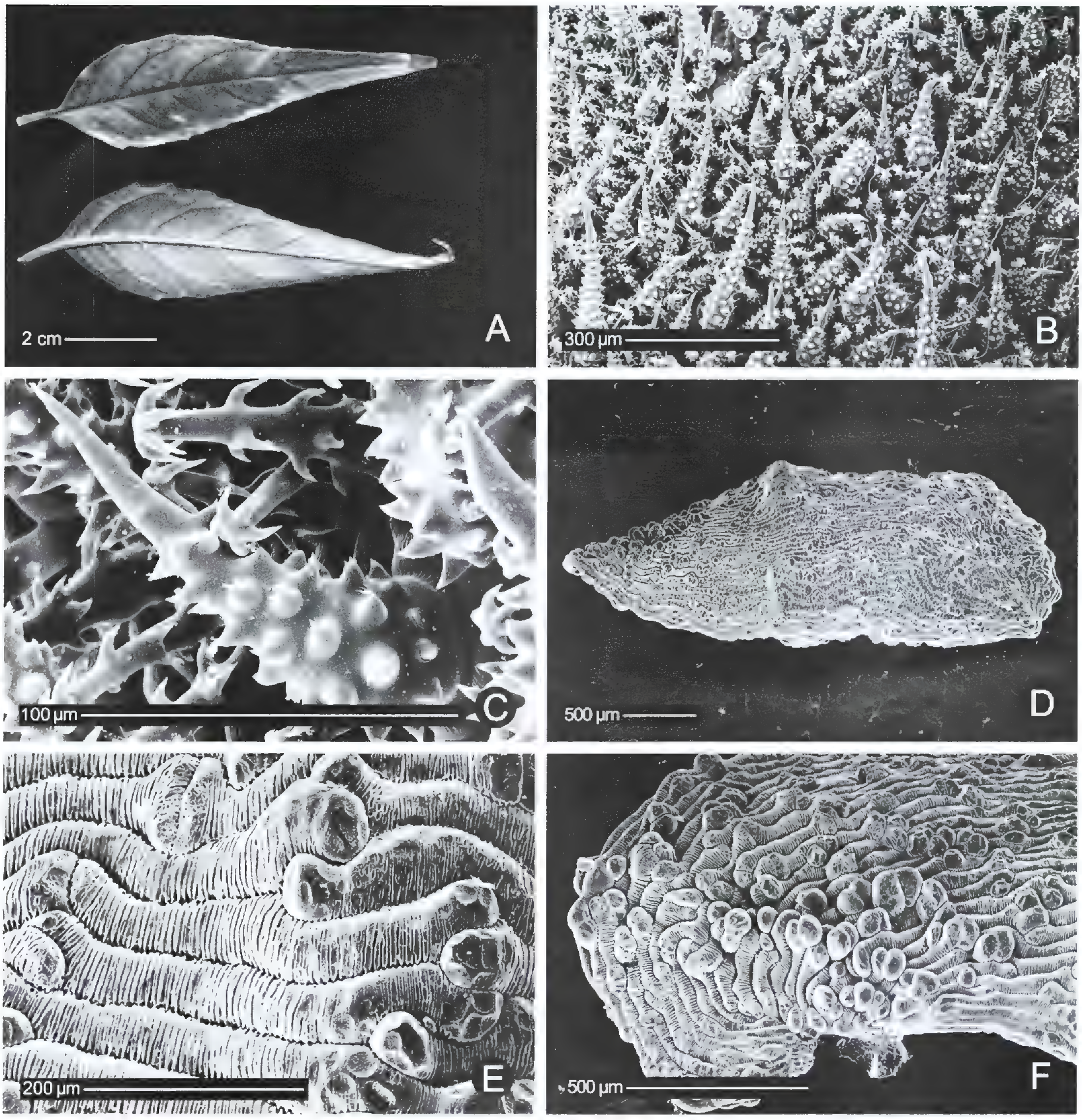


Figure 6. *Mentzelia heterosepala*. —A. Mature foliage leaves (*Weigend & Schwarzer 8027*, cultivated in Berlin). B, C. Indumentum on ovary. —B. Overview, note the very dense cover with short scabrid trichomes. —C. Details, scabrid trichome. D–F. Seeds (*Weigend & Schwarzer 8027*). —D. Overview, note the undulated epidermis surface. —E. Detail of testa epidermis, note the transverse striations and thickened poles of outer periclinal wall. —F. Hilar pole of seed and hilum.

Mentzelia grisebachii Urb. & Gilg, Nov. Actorum Acad. Caes. Leop.-Carol. German. Nat. Cur. 76: 48. 1900. TYPE: Argentina. Catamarca: Río de los Nacimientos, *F. Schickendantz 104* (lectotype, designated here, GOET!).

Mentzelia jujuyensis Sleumer, Bot. Jahrb. Syst. 76(4): 420. 1955. Syn. nov. TYPE: Argentina. Jujuy: Tilcara, Quebrada de Huasamayo, 2600 m, *E. Petersen & J. P. Hjerting 26* (holotype, LIL not seen; isotype, W!).

Annual herb, 0.1–0.5 m tall, divaricately branching; stems basally to 5 mm thick, bark exfoliating on older branches, young shoots densely covered with glochidiate trichomes 0.1–0.15 mm long; roots (in Peruvian populations) conspicuously thickened and succulent, up to 150 × 10–15 mm. Leaves opposite below, alternate above; lower petioles up to 15 mm, upper leaves subsessile; lamina ovate, 30–90 × 20–40 mm, lobulate, deeply lobed to pinnatifid or only deeply and irregularly serrate; base cuneate; apex acuminate. Inflorescences as terminal dichasia or thyrsoids, divaricate (branching angle between paraclades of dichasia 160°–180°); bracts strongly recaulescent on branches (10 mm or to next flower), bracts 15–30 × 5–15 mm. Flowers sessile, with

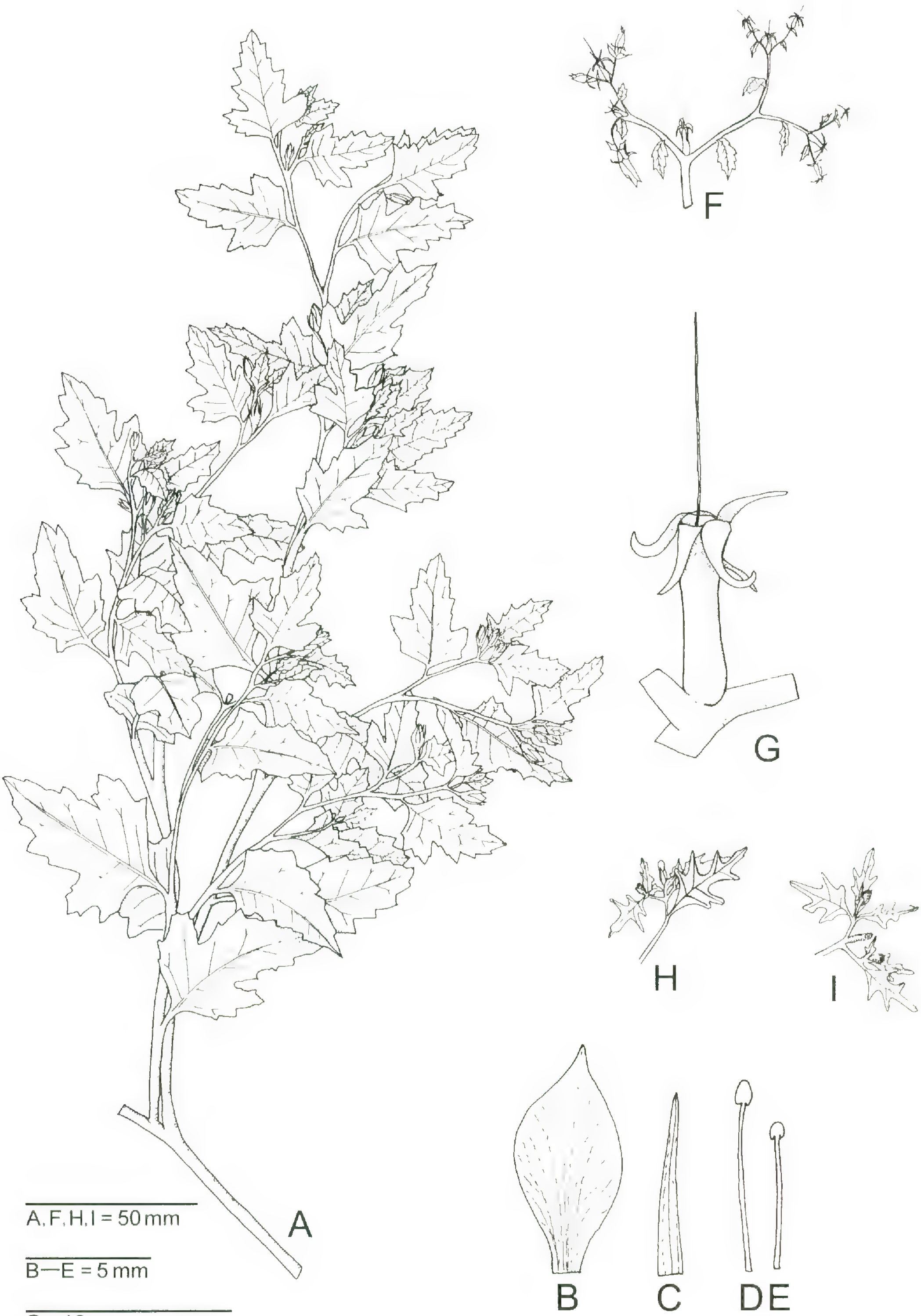


Figure 7. *Mentzelia parvifolia*. —A. Entire shoot with inflorescences. —B. Petal. —C. Calyx lobe. —D. Outer stamen. —E. Inner stamen (*Coro 63*). —F. Inflorescence. —G. Fruit (*Beck 26563*). —H, I. Inflorescence branches (*Spooner 6585*).

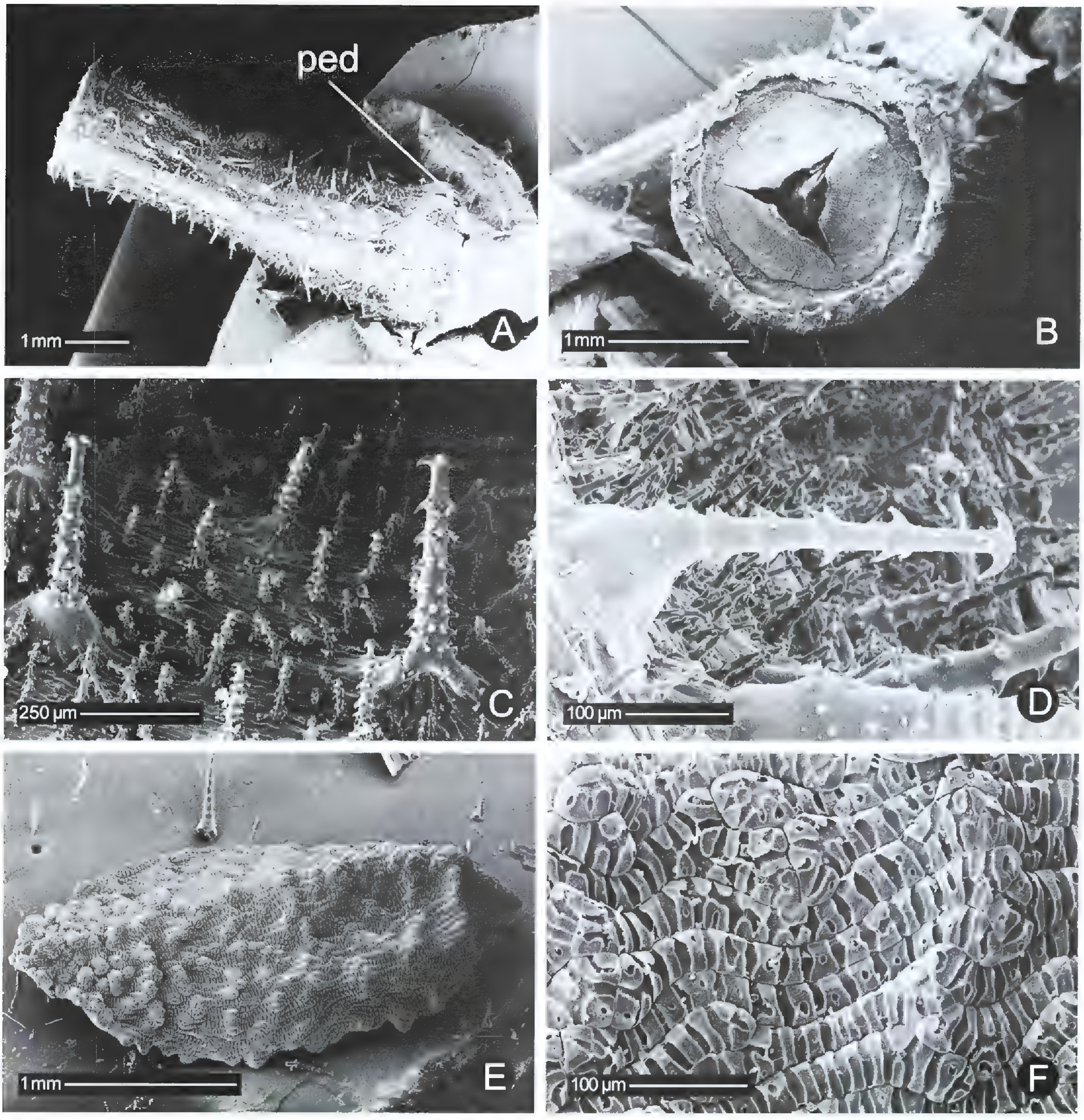


Figure 8. *Mentzelia parvifolia*. A, B. Fruit (*Weigend et al. 8337*). —A. Fruit, lateral view, note indistinct, thickened pedicel (ped). —B. Apex of mature capsule with barely opened apical valves. C, D. Indumentum on ovary (*Coro 63*). —C. Overview, mixture of long and short glochidiate trichomes. —D. Close-up of long glochidiate trichome. E, F. Seed (*Weigend et al. 8337*). —E. Overview, note irregularly angular shape and undulate epidermis. —F. Detail of testa, note transverse grooves and thickened end of testa cells.

cylindrical calyx tube, 4–5 × 1–2 mm, with dense cover of glochidiate hairs 50–500 µm long; calyx lobes long-acuminate from narrowly oblong base, 4–7 × 1–2 mm; petals obovate, 10–15 × 5 mm, base narrowed, apex apiculate, pale yellow. Stamens 35 to 45, ca. 10 mm, anthers ovate to oblong, ca. 0.5 × 0.3 mm, pale yellow; style filiform, ca. 10 mm, with 3 decurrent stigmas. Capsule sessile or shortly pedicellate, erect or more often deflexed, 10–14 × 1.5 mm, covered with glochidiate trichomes only, longer ones ca. 300–450 × 60 µm, with 5 to 7 bands of hooks, shorter ones 60–200 µm, with 3 to 5 bands of hooks; seeds 3 to 6, 2.5–3.5 × 1.5 mm, greyish brown.

In the protologue of *Mentzelia parviflora*, the additional specimens *F. Kurtz 614* (B, CORD) and *F. Kurtz 2724* (B) are cited. *F. Kurtz 614* (B) was selected as lectotype by Sleumer (1955), although only the photograph of the Berlin specimen (B† photo at F 010131) is extant. A new lectotypification is therefore required, and I propose choosing the CORD specimen, since *F. Kurtz 2724* (B) is also lost. The

protologue for *M. grisebachii* (Urban & Gilg, 1900) also cites the specimens *F. Schickendantz 11* (B), *F. Schickendantz 250* (B, B photo at F 10126), *O. Kuntze s.n.* (B), *G. Hieronymus & P. G. Lorentz 326* (G, K, NY), and *C. Spegazzini 104351a* (B). Lectotypification is therefore required. The Berlin material was destroyed by fire in 1943 (Hiepko, 1987) and *G. Hieronymus & P. G. Lorentz 326* is a mixed collection with *M. aspera*; this leaves *F. Schickendantz 104* (GOET) as the only extant and unequivocal collection, and it is therefore used here to lectotypify the species.

The fruits of *Mentzelia parvifolia* (Fig. 13A, B) are slightly variable in shape, and separate taxa have been established for the forms with shortly pedicellate instead of sessile capsules (*M. grisebachii*, *M. parvifolia* var. *transiens*, *M. jujuyensis*; Urban & Gilg, 1900; Sleumer, 1955). However, sessile and pedicellate fruits are frequently found on the same plant, and even the type specimen of *M. parvifolia* var. *transiens* (*Sleumer 2663*, W) has both sessile and pedicellate fruits, so that this character may be disregarded. From my own observations, these characters are continuously distributed and I was unable to recognize discrete taxa.

Two specimens from Bolivia (Cochabamba, *Pedrotti et al. 31*, and Chuquisaca, *Smith et al. 396*) apparently lack the typical divaricate branching pattern and also have unusually long, closely serrate leaf margins and are thus fairly aberrant for the species. More material and mature fruits will be required to ascertain whether these specimens represent a distinct taxon.

Distribution and habitat. *Mentzelia parvifolia* is reported here for the first time from Peru, where it is locally very abundant in a semi-desert area between Omate and Moquegua. It apparently only germinates in unusually moist years and was abundant in April 2006 in places where a single plant could not be found in April 2004. In Peru, where the plant was studied in the field, the plant occurred in mixed stands with, e.g., *Cistanthe celosioides* (Phillipi) Carolin ex Hershk. and *Malesherbia arequipensis* Ricardi.

The species is now known from Peru, Bolivia, and Argentina and has been collected at elevations of (900–)1500–2500(−3500) m. In addition to the provinces recorded below, the species as defined here is also reported from Argentina from the provinces of Jujuy, La Pampa, San Luis, Santiago del Estero, Santa Fé, and Tucumán (Sleumer, 1955).

Additional specimens examined. ARGENTINA. **Catamarca:** Belén, Quebrada de Belén, *Cabrera et al. 16756* (M); Belén, Quebrada de Belén, *Sleumer & Vervoorst 2359* (BR, W); Belén, btw. Pozo de Piedra & Yacutula, Las Faldas, *Sleumer & Vervoorst 2637* (W); Tinogasta, Chaschuil valley, 69 km on Rte. 45 from Fiambalá to Paso San Francisco, *Leuenberger et al. 4239* (B, CORD, Z); Quebrada de Polén to Puerta de San José, *Sleumer & Vervoorst 2360* (W); btw. Granadillas & Pozo de Piedra, *Sleumer & Vervoorst 2385* (W); Lampasito, *Reales 1981* (W); Punta Balestro, O'*Donnell-Meyer 5175* (W); El Desmonte, Punta Balestro to Santa Maria, O'*Donnell-Meyer s.n.* (W); Tinogasta, near El Algarrobal, *Vervoorst 3148* (W); Province Andalgalá, Cuesta de Chilca, *Hunziker & Gamerro 12434* (MO); Dpto. Andalgalá, La Playa, *Jörgensen 916* (MO). **Córdoba:** Dpto. Pumilla, Calera, *Stuckert 12440* (G); s. loc., Dec. 1891, *Kuntze s.n.* (NY); Tulumba, rd. 16, 27 km W of Dean Funes, *Krapovickas & Cristóbal 46594* (MO). **La Rioja:** Capital, Ruta Provincial 10, Km 891, Sierra de Mazan, *Hunziker & Gamerro 12488* (MO); Chilecito, *Dawson 3428* (HUT); Famatina, RN 40, *Botta & Miconi 598* (MO). **Mendoza:** Las Heras, Challao, *Paci & Barkley 19Ar902* (W). **Salta:** Guachipas, Arroyo Alemania, N of railroad bridge, *Novarra 8443* (Z); Cafayate, Yacuchuya, *Castellanos 46850* (W); Campos de Cachi, *Castellanos 46857* (W); Gólgota, *West 6181* (MO); Santa Victoria, Santa Victoria, *Meyer 5260* (W); Rosario de Lerma, Santa Rosa de Tastil, rte. 51, Pucará, *Nicora et al. 8985* (MO). **San Juan:** Jachal, Cuesta del Huaco, *Palacios et al. 1706* (BR, W); Ullún, path to Quebrada de la Dehesa, *R. Kiesling et al. 5949* (MO); Quebrada de Talacasto, *Cabrera 30166* (MO). BOLIVIA. **Chuquisaca:** Sud Cinti, Tajsara mtns., *Gerold 33* (LPB, M). **Cochabamba:** Capinota, near Capinota, *Pedrotti et al. 31* (LPB). **Potosí:** Nor Chichas, 5 km N of Orhcolu, *Torrico & Peca 496* (LPB, M, MSB). **Tarija:** Cercado, btw. Tarija & Tanibut, *Bastián 685* (LPB, M); Santa Ana, *Coro 1248* (LPB, M); Cercado, path to Yesera, 6 km, *Ehrich 469* (LPB, M); Cercado, 10.2 km N of toll station 6 km E of Tarija, rd. to Entre Rios, *Spooner & García 6585* (LPB, TAES, WIS); Méndez, *Coro 63* (LPB). **Province unknown:** Bolivian plateau, *Bang 1005* (E, G, M, W), *Bang 748* (W). PERU. **Moquegua:** Mariscal Nieto, rd. Omate to Moquegua, 2 km after bridge over Río Tambo, *Weigend et al. 8349* (B, HUSA, USM); Mariscal Nieto, rd. Omate to Moquegua, 4 km after bridge over Río Tambo, *Weigend et al. 8361* (B, HUSA, USM);. General Sánchez Cerro, btw. Omate & Moquegua, directly beneath Omate, Urinay, *Weigend et al. 8337* (B, HUSA, USM).

7. **Mentzelia scabra** Kunth in HBK, Nov. Gen. Sp. 6: 120. 1823. TYPE: [Colombia. Nariño:] btw. Pasto & Tuquerres, *A. Humboldt & A. Bonpland 2173* (lectotype, designated by Weigend, 2001: 25, P-BONPL!; isotype, P!).

Strongly branched subshrub or shrub, 2 m tall, erect, more rarely decumbent or ascending or climbing; stems basally 5–20 mm thick, lignescent or ligneous, very brittle, with white to beige exfoliating bark or persistent, dark brown to black bark with conspicuous lenticels; young shoots very densely set with glochidiate and scabrid trichomes. Leaves opposite on seedlings, then spirally inserted, subopposite or opposite in the inflorescence; petioles 2–15 mm; lamina ovate, 20–150 × 35–70 mm, lobed or undivided, usually with 1 distinctive leaf lobe proximally on each side, margin irregularly serrate to lobulate; base shallowly cordate, truncate to

cuneate; apex always acuminate. Inflorescences as terminal, frondose thyrsoids, widely branched (branching angle between paraclades of dichasia < 90°); bracts 10–60 × 10–35 mm; pedicels 1–5 mm. Flowers with calyx tube conical to ovoidal, ca. 5 × 3 mm, densely covered with glochidiate and scabrid trichomes 0.1–2.5 mm long; calyx lobes acuminate from ovate base, 7–15 × 1–3 mm; petals widely obovate, apex apiculate, 12–30 × 6–16 mm, slightly pilose apically on back, golden yellow to pale yellow; stamens 60 to 120, ca. 10 outer ones usually ca. 1.5–2× as long as inner ones and erect from a spreading base, the inner ones shorter and negatively geotropic; anthers ca. 1 × 0.75 mm, pale yellow, style filiform with 3 decurrent stigmas. Capsule obovoid to clavate, with pedicel (0–)4–10 mm, opening with 3 apical valves, 8–14 × 5–7 mm, covered with a mixed indument of (at least basally) erect to slightly acroscopic scabrid and glochidiate trichomes, scabrid trichomes ca. 3–4× longer than glochidiate trichomes; seeds 1 to 5, brown, irregularly oblong, ca. 4.5 × 1.8 mm, grey.

Mentzelia scabra is redefined here as a widespread and rather heterogeneous species including the majority of species previously recognized from *Mentzelia* sect. *Mentzelia* in South America (*M. soratensis* Urb. & Gilg, *M. fendleriana* Urb. & Gilg, *M. chilensis* Gray, *M. cordobensis* Urb. & Gilg, and *M. grandiflora* Ruiz & Pav. ex G. Don). Although there is no particularly clear dividing line between *M. scabra* from South America and *M. hispida* in northern Central America, fruit size, pedicel length, and the differences in trichome complement indicate that they are distinct entities. The otherwise very similar Mexican/North American *M. hispida* has capsules 2.5–5× as long as wide (vs. 1.5–2× as long as wide) and often very long-pedicellate (up to 15 mm, *W. Boege 184 & 1445*, *M. Rosas R. 61* [all FR]). Four of the species described for South America can be assigned subspecies rank on the basis of relatively weak but constant characters (habit, flower size, bract size, and indument on the ovary) and are here recognized as *M. scabra* subsp. *chilensis*, subsp. *grandiflora*, subsp. *scabra*, and subsp. *cordobensis*. All of these subspecies are relatively narrowly endemic, apart from subspies *chilensis* (see that section for discussion), which covers nearly the entire distributional range of the species. *Mentzelia cordobensis* from Argentina is only marginally different from typical *M. scabra* from southern Colombia and differs in minor details of the length and composition of the indument and in leaf division and plant habit. It is reduced here to subspecies rank, since there are some, albeit weak, differentiating characters and more or less exclusive distribution areas. Also, *M. chilensis* var. *atacamensis* Urb. & Gilg is included here in *M. scabra* as a subspecies because its key differentiating character (all stamens are approximately equal in size) is also found in other specimens of *M. scabra*, but its small flowers, nearly spheroidal fruits, and very long internodes are sufficiently distinct to segregate it. The typical subspecies, subspecies *scabra*, is a shrub with persistent, dark bark. All other subspecies are subshrubs, with branches only occasionally persisting for more than one year, and all of them have pale, exfoliating bark. The only exception is a limestone population of *M. scabra* subsp. *chilensis* from the province Chota in northern Peru, which is also shrubby, but differs in its much smaller flowers and leaves. *Mentzelia scabra* subsp. *chilensis* and *M. scabra* subsp. *atacamensis* are apparently at least facultatively annual. Even after eight years in cultivation, plants of *M. scabra* subsp. *chilensis* have not produced a persistent bark and have retained their subshrubby habit (*Weigend & Dostert 97/144b-C*, cultivated in Munich 1997–2000 and in Berlin 2000–2005).

Key to the Subspecies of *Mentzelia scabra*

1a. Stamens of equal length (differing in length up to 1 mm); upper bracts subcircular to widely ovate, 5–15 mm diam.; petals 12–15 mm (only northern Chile) . . . . . 7.1. *M. scabra* subsp. *atacamensis*

1b. Stamens distinctly unequal, outer ones 1.5–2× longer than interior ones; upper bracts ovate to narrowly ovate, over 15 mm diam.; petals (15–)18–30 mm long (northern Chile, Argentina, north to Venezuela).

2a. Capsules subsessile, ca. 14 mm long, with very long scabrid trichomes (1.5–2 mm); lamina (55–)70–120 mm, indistinctly lobed, coarsely serrate . . . . . 7.2. *M. scabra* subsp. *boliviana*

2b. Capsules usually less than 10 mm long, pedicellate if longer, with scabrid trichomes less than 1 mm long; lamina less than 55 mm long, if longer then distinctly lobed and/or shallowly serrate.

3a. Inflorescence lax, i.e., internodes 1–7× as long as subtending bracts . . . . . 7.3. *M. scabra* subsp. *chilensis*

3b. Inflorescence dense, i.e., internodes less than 1.5× as long as subtending bracts.

4a. Petals (10–)12–15 mm long (Argentina) . . . . . 7.4. *M. scabra* subsp. *cordobensis*

4b. Petals 20–30 mm long (Colombia and Peru).

5a. Subshrubs with pale, exfoliating bark, stems basally less than 10 mm thick; petals 18–25 mm long; flowers with 60 to 70 stamens (only central Peru) . . . . . 7.5. *M. scabra* subsp. *grandiflora*

5b. Shrubs with dark, lenticellate bark on basal stems, these 10–20 mm thick; petals 25–30 mm long; flowers with 100 to 120 stamens (only southern Colombia) . . . 7.6. *M. scabra* subsp. *scabra*

7.1. Mentzelia scabra Kunth subsp. **atacamensis** (Urb. & Gilg) Weigend, comb. et stat. nov. Basionym: *Mentzelia chilensis* Gay var. *atacamensis* Urb. & Gilg, Nov. Actorum Acad. Caes. Leop.-Carol. German. Nat. Cur. 76: 50. 1900. TYPE: Chile. Atacama: Atacama, *F. Philippi 323* (lectotype, designated here, W!; isotypes, B†, SGO not seen). Figure 9.

Subshrub ca. 0.5 m tall; stem 4–8 mm thick basally, with pale, exfoliating bark. Leaves subsessile or shortly petiolate (lower leaves with petioles 5–10 mm); lamina widely ovate to subcircular, 15–25(–35) × 10–15(–22) mm, margin very shallowly lobulate to serrate (lobules usually less than 2 mm deep); base slightly decurrent on petiole, lower leaves with rounded base, upper with subcordate base. Inflorescences very lax; internodes 3–7× as long as subtending bracts; pedicels 2–5(–8) mm. Flowers with calyx tube covered with short glochidiate trichomes (0.1–0.5 mm) and longer scabrid trichomes (≤ 0.5 mm); calyx lobes 8–10 × 2–4 mm; petals ca. 15 × 8–10 mm; stamens 40 to 50, filaments 10–11 mm, all ± equally long. Capsule ovoidal, 5–8 × 5 mm.

Both the B and the W sheets were annotated by Urban and Gilg; the W collection, as the only extant set, is therefore chosen as lectotype. *Mentzelia scabra* subsp. *atacamensis* differs from the other subspecies of *M. scabra* in its subequal stamens, and this, together with generally less deeply lobed and smaller leaves, the very long internodes in the inflorescence, and the small flowers, was also the reason why Urban and Gilg (1900) segregated it as a variety. The specimens of *M. scabra* subsp. *chilensis* with subequal stamens from northern Peru clearly refer to subsp. *chilensis* because of their lobed leaves and narrower capsules, but their existence is the reason for not recognizing *M. scabra* subsp. *atacamensis* at species level.

Distribution and habitat. This subspecies is known from Regions II and IV in Chile and is also to be expected in Region III. It grows at elevations of 30–1000 (possibly to 3000) m, and is likely a faculative annual.

Additional specimens examined. CHILE. **II Región, de Antofagasta:** Taltal, Taltal, *Werdermann 798* (B, E, G, M, MO, Z), *von Bayern 198* (M); 12 km SE of Taltal, *Charpin et al. 1C23612* (G); Quebrada Peralito, *Quezada & Ruiz 392* (M); Lomas de Taltal, 7 km S of Taltal, *Ackermann 481* (BSB, CONC, M, SGO); Cobija, *Gaudichaud s.n.* (G); Antofagasta, Quebrada Peralito, Paposo, 1.5 km N of Taltal, *Billiet & Jadin 5545* (MO); near Taltal, *Zöllner 14286* (MO); 7 km E of Taltal, *Eggli & Leuenberger 1766* (B). **IV Región, de Coquimbo:** Elqui, Los Chiches, 3 km W of Vicuña, *Wagenknecht 18449* (G, MO), *Worth & Morrison 16344* (G, MO); Elqui, Punta N of Cerro Los Mantos, *Mahu s.n.* (MO); Illapel, *Geisse s.n.* (Z); Andacollo valley, *Elliot 31* (E).

7.2. Mentzelia scabra Kunth subsp. **boliviana** Weigend, subsp. nov. TYPE: Bolivia. Cochabamba: Ayopaya, 2500 m, *S. G. Beck & R. Seidel 14597* (holotype, LPB!; isotype, M!). Figures 10, 11A–E.

Haec subspecies *Mentzeliae scabrae* subsp. *chilensi* affinis, sed ab ea ovario trichomatibus longioribus usque ad 2 mm longibus instructo et habitu frutice maiore, foliis et floribus maioribus differt.

Shrub 0.6–1.2 m tall; stems 5–15(–20?) mm thick basally, with pale, strongly exfoliating bark. Leaves subsessile to shortly petiolate (lower leaves with petioles 3–7 mm), (55–)70–120 × 30–60 mm, not or very shallowly lobed, margin mucronate to serrate; base subcordate to subcuneate; apex acuminate. Inflorescences dense, internodes usually much shorter than subtending bracts; bracts ovate, 35–65 × 10–35 mm; pedicels 1–3 mm. Flowers with calyx tube with short glochidiate and scabrid trichomes to 2 mm long, calyx lobes 13–17 × 3–5 mm; petals ca. 25 × 20 mm; stamens 70 to 90, 10 outer ones markedly longer, 20–25 mm, inner ones only 10–15 mm. Capsule narrowly clavate, 14–17 × 5–6 mm, covered with scabrid and glochidiate trichomes, scabrid trichomes 1500–2500 µm long, glochidiate trichomes ca. 150 (rarely to 600) µm long.

Mentzelia scabra subsp. *boliviana* is readily recognized by its shallowly lobed or unlobed leaves with coarsely serrate margin (Fig. 10A) and especially by the long scabrid trichomes with a smooth apex on its ovaries and fruits (Fig. 11A–E). These trichomes differ both in length (1.5–2 mm vs. less than 1 mm in other subspecies) and apex (smooth vs. scabrid in other subspecies). The taxon is relatively widespread in Bolivia but has not yet been collected in adjacent Argentina, where it is replaced by *M. scabra* subsp. *cordobensis*, which has much smaller flowers and different indument on its ovaries.

Distribution and habitat. This subspecies is known only from Bolivia and grows in four of its departments at elevations of 2400–3200 m. It is probably a robust subshrub similar to *Mentzelia scabra* subsp. *chilensis*, from which it differs in both leaf size and ovary indument.

Paratypes. BOLIVIA. **Chuquisaca:** Nor Cinti, La Palma to El Chaco, Río Chico valley btw. Sucre & Puente Arce, *J. R. I. Wood 8112* (LPB, M); Nor Cinti, rd. Padcoyo–Calapaya

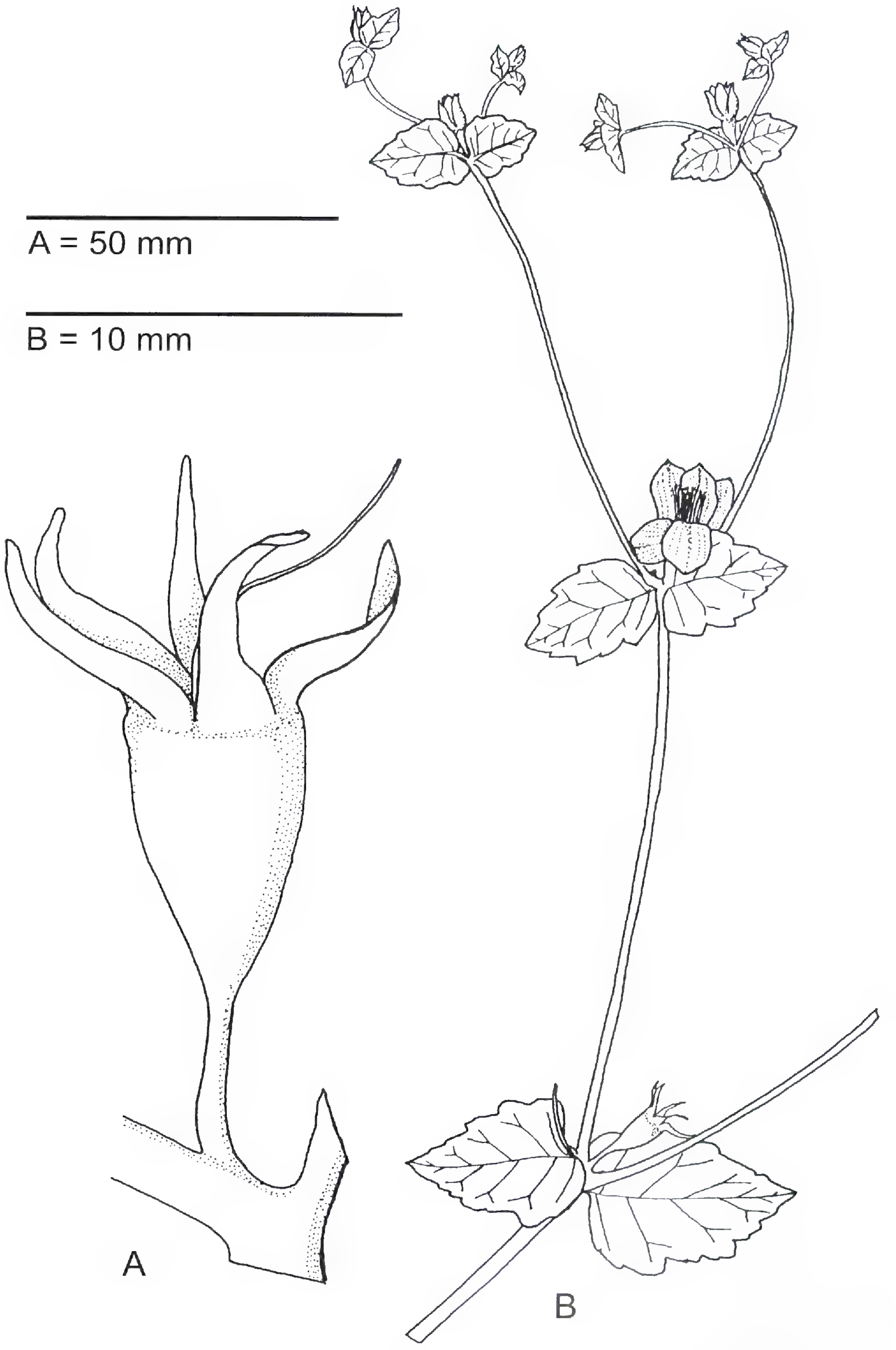


Figure 9. *Mentzelia scabra* subsp. *atacamensis*. —A. Inflorescence. —B. Young fruit. Drawn from *Werdermann 798*.

Figure 10. *Mentzelia scabra* subsp. *boliviana* Weigend. —A. Inflorescence (drawn from *Wood et al. 15337*). —B. Fruiting branch. —C. Fruit (drawn from *Cárdenas 6082*).

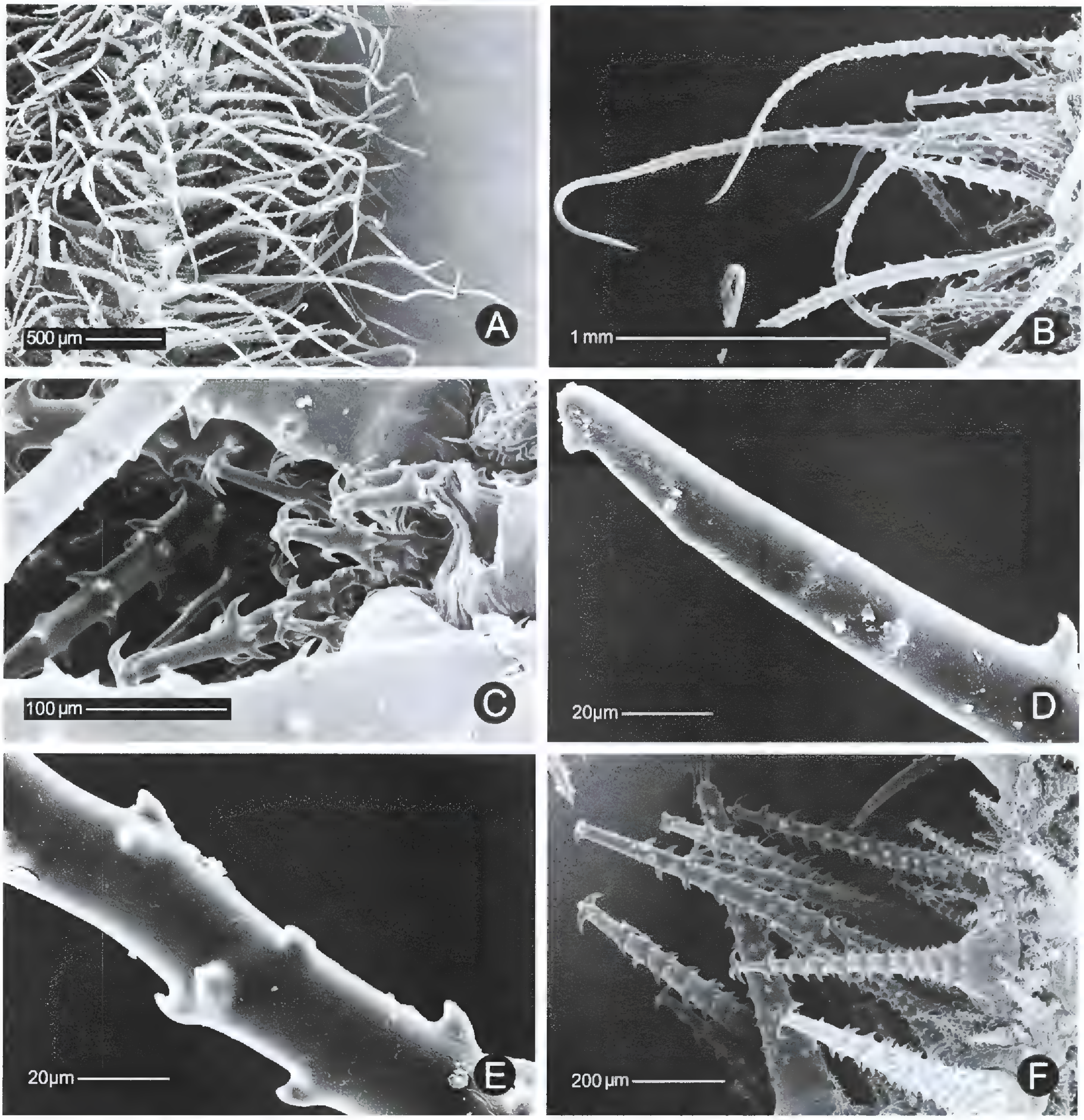


Figure 11. *Mentzelia scabra* subsp. *boliviana* Weigend. A–E. Indumentum on ovary. —A. Mixed indumentum showing very long scabrid trichomes and very short glochidiate trichomes. —B. Long scabrid trichomes and shorter glochidiate trichomes. —C. Apex of scabrid trichome. —D. Median portion of scabrid trichome. —E. Very short glochidiate trichome. —F. *Mentzelia hispida.* Mixed indumentum of long and short glochidiate trichomes. A from *Ugent et al. 4997*; B–E from *Troll 1170*; F from *Flügel 9025*, FR.

Betanazos, btw. Calapaya & Betanazos, *S. D. Smith & S. Leiva G. 411* (F, HAO, LPB, MO); Azurduy, 10–15 km S of Tarvita on rd. to Sopachuy, *J. R. I. Wood, D. J. Goyder & M. Serrano 15337* (LPB). **Cochabamba:** Arque, Río Arque, btw. Arque & Iguerani, *P. Ibisch 156* (LPB); Ayopaya, *S. G. Beck & R. Seidel 14597* (LPB, M); Mizque, path Chaguarani to Mina Asientos, Comunidad San Vicente, *A. López & E. Saravia 402* (Cochabamba); Mizque, Quioma Silver Mines, *Eyerdam 25337* (M); Cercado, Quebrada Chuncado, *N & M. Ritter 2973* (MO). **Sucre:** Rd. Sucre to Cochabamba, Km 25, *M. Cardenas 6082* (WIS); Oropeza, Sucre–Aiquile rd., 15 km ENE of Sucre, *D. & V. Ugent & M. Cardenas. 4997* (K, WIS). **Tarija:** Prov. Arce, La Merced, *R. Ehrich 492* (LPB). **Province unknown:** Pucarasito, *C. Troll 1170* (M).

7.3. Mentzelia scabra Kunth subsp. **chilensis** (Gay) Weigend, comb. et stat. nov. Basionym: *Mentzelia chilensis* Gay, Fl. Chil. 2: 431. 1863. TYPE: Chile. Coquimbo: Mtns. of Arqueros, Piedra Colgada, *C. Gay 265* (lectotype, designated here, P!; isotypes, P[3]!). Figures 12A–F, 13.

Mentzelia ignea (Phil.) Urb. & Gilg in Engl. & Prantl, Nat. Pflanzenfam. 3(6a): 110. 1894. Syn. nov. Basionym: *Loasa ignea* Phil., Anales Mus. Nac. Santiago de Chile [Verz. Antofagasta Pfl.]: 24. 1891. TYPE: [Chile. Tarapacá:] btw. Mocha & Guaviña, *F. Philippi s.n.* (holotype, SGO not seen; isotype, K!).

Mentzelia fendleriana Urb. & Gilg, Mem. Torrey Bot. Club 3: 34. 1893. Syn. nov. TYPE: Venezuela. Miranda: "prope coloniam Tovar," *A. Fendler 1876* (lectotype, designated by Weigend, 1996b: 237, G!; isotypes, GH not seen, GOET!, MO not seen).

Mentzelia soratensis Urb. & Gilg, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 76: 68. 1900. Syn. nov. TYPE: Bolivia. [La Paz: Larecaja:] near Sorata, Munaypata, 2650 m, *G. Mandon 621* (lectotype, designated here, BR!; isotypes, G-DEL!, GH not seen, NY!, P!, W!).

Subshrub, rarely shrub or shrubby annual, usually stiffly erect, rarely decumbent or climbing, 0.6–2 m tall; stems 5–20 mm thick basally; bark thin, pale grey or brown, strongly exfoliating in thin strips, rarely dark brown and persistent. Leaves subsessile to shortly petiolate (lower leaves with petioles 5–10 mm); lamina ovate, 15–75(–90) × 10–50(–65) mm, with 1–(2–3) ± distinct lobes on each side (unlobed), lobes triangular, triangular-ovate, or oblong, margin serrate; base subcordate to cuneate. Inflorescence lax, rarely dense; internodes 1–7× as long as subtending bracts; bracts ovate; pedicels 1–2(–4) mm. Flowers with calyx tube densely set with glochidiate and scabrid hairs 0.1–0.8(–1) mm long, longest hairs scabrid; calyx lobes 8–10(–20) × 2–3(–5) mm; petals (10–)14–18(–25) × (7–)10–12 (–18) mm; stamens (50 to)70 to 90(to 100), 10 outer ones markedly longer, (8–)14(–20) mm, inner ones only (5–)6–11(–15) mm. Capsule obovoid, 6–12(–17) × 5–6(–7) mm, base rounded or conical, covered with scabrid and glochidiate trichomes, scabrid trichomes from 400–500 µm ("Cordillera Negra"), 600–750 µm (most specimens), to 800 µm long ("*Mentzelia soratensis*").

Four Gay specimens are deposited at P, and lectotypification is required for *Mentzelia chilensis*. One of the specimens is selected here. For *M. soratensis*, two different collections are cited by Urban and Gilg (1900). The best-preserved specimen (*Mandon 621*, BR) is selected here as the lectotype. Urban and Gilg (1900) also cited *Pentland s.n.* as syntype for *M. soratensis*, but I failed to locate the original material, which may have been housed in Berlin only. *Mentzelia scabra* subsp. *chilensis*, as understood here, includes *M. fendleriana*, *M. soratensis*, and *M. ignea*. The types of *M. chilensis* and *M. ignea* are from the same area in northern Chile, and two of the syntypes of *M. fendleriana* (*Bang 748, 1082*; see Weigend, 2000) and the types of *M. soratensis* are from the same area in Bolivia. The various species as recognized by Urban and Gilg (1900) and Darlington (1934) had very peculiar distribution patterns: *M. soratensis* was described from Bolivia by Urban and Gilg (1900) and reported from Ecuador, Peru, and Bolivia by Darlington (1934), whereas the syntypes of *M. fendleriana* came from Venezuela and Bolivia, and Darlington (1934) subsequently reported the species from Venezuela, Ecuador, Peru, and Bolivia (i.e., both species appeared to have fully overlapping geographical ranges). *Mentzelia ignea* was described from Chile but reported from Chile and Peru by Urban and Gilg (1900), and their definition included material that was subsequently referred to *M. cordifolia* by Darlington (1934), whereas Darlington reported material of *M. cordobensis* (= *M. scabra* subsp. *cordobensis*) as belonging to *M. chilensis*. Thus, the confused taxonomic history corroborates that there may be no clear morphological characters that allow a segregation of the various species previously recognized. All my attempts to find any clear dividing lines among any of these taxa failed.

Additional specimens examined. BOLIVIA. **La Paz:** Bolivian Plateau, *Bang 748* (E, MO, NY), *Bang 1082* (NY); Larecaja, from Sorata toward Río San Cristóbal, *Beck 18724* (LPB, M); Larecaja, Sorata, *Troll 2413* (B, M); Larecaja, from Larecaja 10 km toward La Paz, *Beck 8682* (LPB, M); Larecaja, SE of Sorata, *Jardim 312* (MO); Murillo, SSE of La Paz, rd. to Nevado Illimaní, from Palca 20 km toward Cohoni, *Beck 8403* (LPB, M). **Potosí:** Bautista Saavedra, Km 61 above Millases on rd. to Betanzos, *Wood 11252* (LPB). **Santa Cruz:** Florida, rd. Mairana to Yunga de Mairana, 2 km NE of Plaza Mayor, *Nee 49332* (BSB, NY); Cordillera, Estancia Rancho Chico, *Fuentes 2378* (MO). **Tarija:** E of Tarija, *Fiebrig 2662* (G); Arce, La Merced, *Ehrich 138* (LPB, M). CHILE: **IV Región, de Coquimbo:** Ovalle, Río Ponio, *Jiles 1888* (CONC, M). COLOMBIA. **César:** Serranía de Perijá, Mpio Augustin Codazzi, *Rangel et al. 12301* (COL, M). ECUADOR. **Azuay:** 30 km S of Santa Isabel, rd. Pasaje to Santa Isabel to Girón, *Harling & L. Andersson 14443* (GB); rd. Pasaje to Santa Isabel, *Harling 25700* (GB); bridge over Río Leon, *MacBryde 343* (QCA); S of Río León valley, *Wiggins 10847* (MO). **Chimborazo:** Canyon of Río Chanchan near Huigra, *Camp E-3191* (P); Chunchi to Sibambe, *Faegerlind & Wibom 744* (S). **Imbabura:** Rd. Pimampiro to Shanshipampa, *van der Werff & Gudiño 10958* (GB); N of Salinas, N of Ibarra, *Juncoso 2232* (QCA); Salinas, *Harling 4333* (S); valley of Río Ambi, *Balslev & Quintana 955* (MSB, NY); btw. Salinas & Carchi, *Holm-Nielsen & Jaramillo 28890* (MO). **Loja:** S of Algarroba, Catamayo valley, *Øllgard et al. 71243* (LOJA, QCA); La Toma to Loja, Km 10, *Madsen 75044* (LOJA, QCA); Río Comunidades, Vilcabamba to Yangana, *Sparre 16136* (S). **Pichincha:** Rd. Guallatubo to Tabacundo, bridge over Río Pisque, *Laegard 51844* (QCA, QCNE); rd. Guayllabamba to Tabacundo at bridge over Río Pisque, *Laegard 51844* (QCA); Perucho, slope above village, *Asplund 20333* (S). **Tunguragua:** Village of Pishilita, next to rd., *Arguello 269* (NY, QCA); valley of Río Píllaro btw. Píllaro & Ambato, *Asplund 8157* (GB, QCA, S, US); above Ambato, *Balls 7148* (E), *Cerón 5890* (QCNE). **Province unknown:** Andes of Ecuador, *Pearce anno 1862* (K). PERU. **Amazonas:** Distr. de Luya, Santo Thomas, Quillay, *Rojas & Vasquez 803* (BSB, MO); Bagua, btw. Bagua Chica & Limonyacu, *Ferreyra 13651* (MO, MSB, USM); btw. Bagua Chica & La

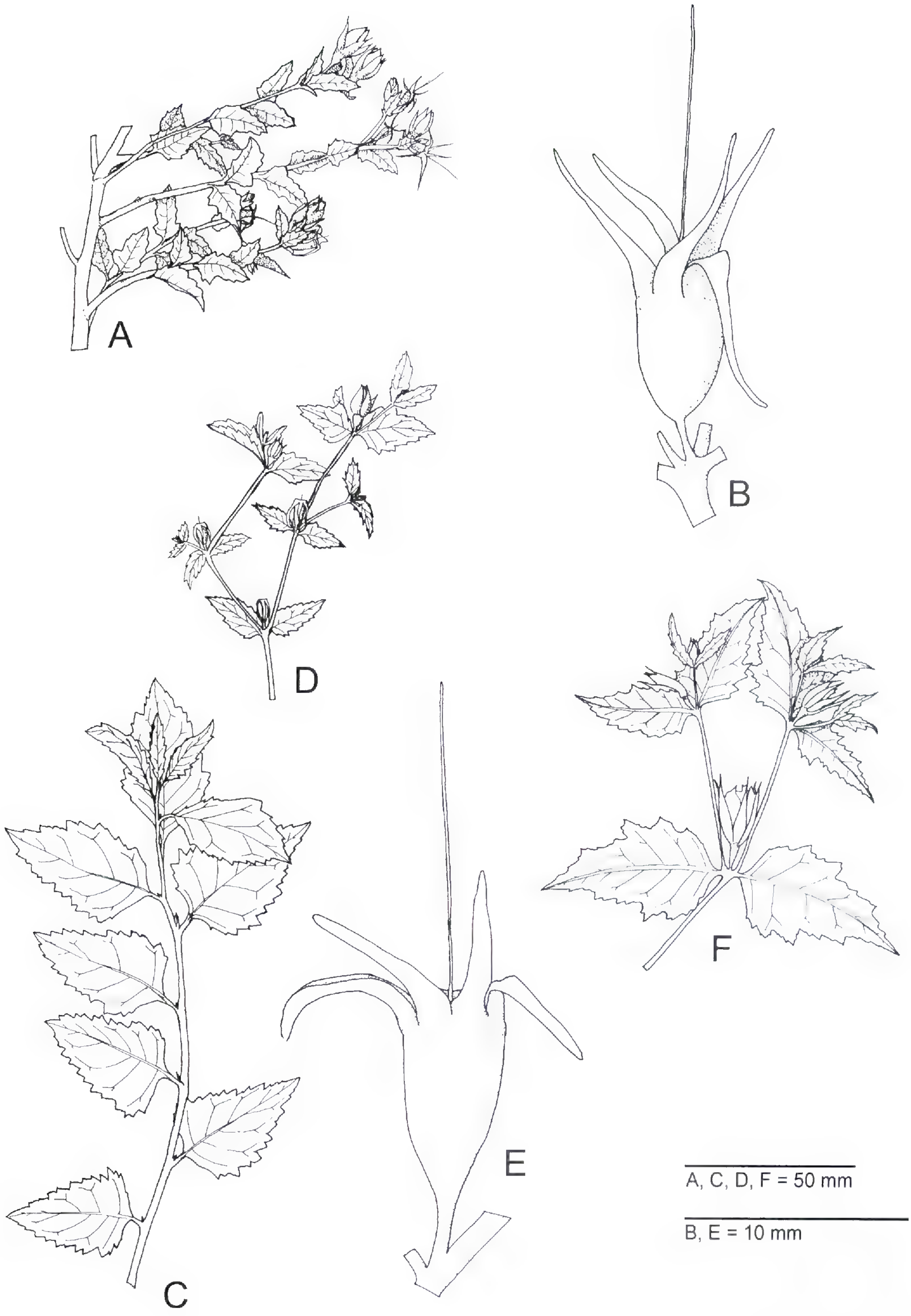


Figure 12. *Mentzelia scabra* subsp. *chilensis*. —A. Flowering shoot, "Chota" form (Peru, Dpto. Cajamarca, Prov. Chota, *Weigend & Dostert 98/142*). —B. Fruit (*Weigend & Dostert 98/142*). C–E. "Cordillera Negra" form (*Weigend & Dostert 98/144b*). —C. Young shoot with serrate leaf margins but without distinct leaf lobes. —D. Inflorescence. —E. Fruit. —F. Inflorescence, individual with serrate leaf margins ("*M. fendleriana*," Venezuela, *Rangel 12301*).

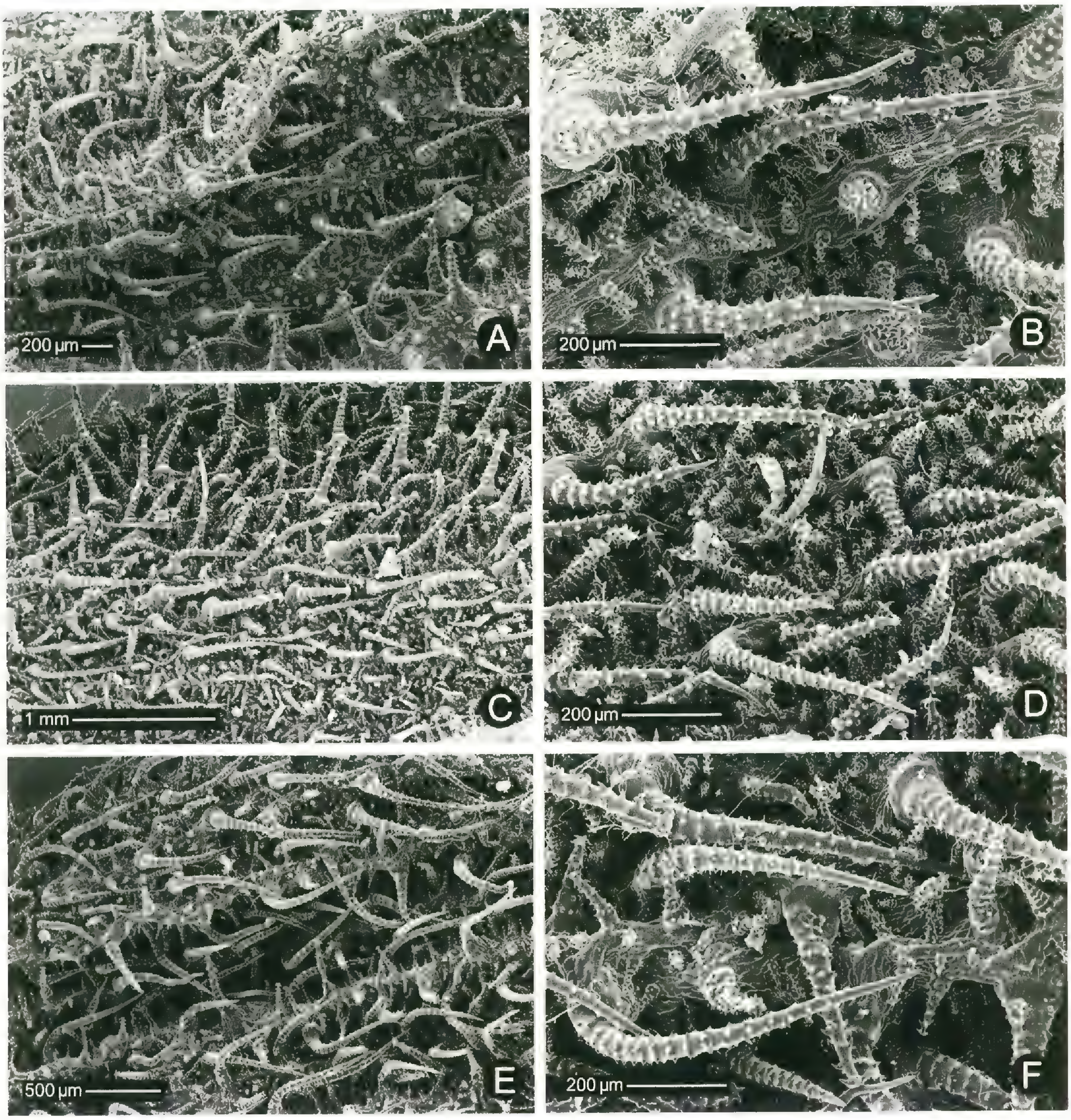


Figure 13. *Mentzelia scabra* subsp. *chilensis* indumentum on ovary and seed. A–B. "Cordillera Negra" form (*Carillo 648*). —A. Overview, mixed indumentum of long scabrid and shorter glochidiate trichomes. —B. Close-up of glochidiate and scabrid trichomes. C–D. "*M. soratensis*" (*Weigend & Weigend 2000/171*). —C. Overview, mixed indumentum of very long scabrid and short glochidiate trichomes. —D. Apex of scabrid trichome. E–F. Seed (*Weigend & Dostert 97/144b*). —E. Overview, note the irregularly angular shape. —F. Detail of testa, note the indistinct transverse striations and slightly thickened ends of the cells.

Peca, 1 km from Bagua, *Dostert 98/87* (CPUN, F, MSB, USM); Camp. Milagro, *Tillett 672-161* (USM); Chachapoyás, rd. Balsas to Leymebamba, *Weigend et al. 98/315* (CPUN, F, M, USM); Chachapoyás, Chachapoyás city, *Weberbauer 4360* (G, USM); near Chachapoyás, *Ferreyra 14426* (MSB, USM); rd. Leymebamba–Chachapoyás, 7 km from Tingo toward Chachapoyás, *Weigend et al. 98/385* (CPUN, F, M, NY, USM); from Leymebamba 3–10 km to Chachapoyás, *Dostert 98/41* (CPUN, F, MO, MSB, NY, USM, W); from Tingo 2–10 km on rd. to Chachapoyás, *Dostert 98/13* (CPUN, F, MSB, USM), *Dostert 98/44* (CPUN, F, MSB, USM); rd. Leymebamba–Chachapoyás, 10 km from Leymebamba toward Tingo, *Weigend et al. 98/375* (CPUN, F, M, NY, USM); Chachapoyás, on dry slope overlooking Río Utcubamba, *Hutchison & Wright 4979* (F, K, M, MICH, MO, NY, P, US, USM); 1 km from turnoff on Tingo–Kuelap rd., *Sánchez & Dillon 9074* (CPUN, F, MO, MSB); rd. Chachapoyás to Mendoza, 13 km from Chachapoyás, *Weigend et al. 98/389* (CPUN, F, M, NY, USM). **Ancash:** Carlos Fitzcaraldo, Yauya, rd. to San Francisco, *Cano et al. 14488* (B, USM); Huari, near Pontó, *Cano et al. 14920* (BSB, USM); Corongo, rd. Sihuas to Corongo–Mirador (Río Santa), *Weigend & Schwarzer 8015* (BSB, HUT, USM); Huraz, Río Grande/Río Chaccan, near Pariacoto, *Weigend et al. 7695* (BSB, HUT, M, USM); Huaraz, below Punta Caillán on rd. to Casma, *Ferreyra 14645* (MSB, USM); Santa, Lomas de Lupín, *Ferreyra 13533*

(USM); Pallasca, Conchucos, *Cano et al. 15563* (BSB, USM); Yungay, Shupluy, path to Guitarrero, *Cano 11330* (USM); Huaylas, Huata, rd. to Pamparomas, *Cano 11060* (USM); Huaylas, 5 km from Caraz on rd. to Pamparomas–Moro, *Weigend et al. 7659* (BSB, HUT, M, USM); Huaylas, Incapamanan, *Carrillo 648* (MSB, USM); Pueblo Libre, *Cano et al. 10977* (USM); Huaylas, Ullpan, Pamaparomas, *Albán 6809* (M, USM); rd. Pamaparomas to Pampap, *Weigend & Dostert 97/144b* (F, HUT, K, M, MO, NY, USM, W); seeds from previous locality, cultivated at Munich, *Weigend & Dostert 97/144b-C* (BSB, M); opposite Mancos, Cerro Negro, *Smith & Bas 4870* (USM). **Apurímac:** Abancay: 35 km SW of Abancay, Hac. Casinchihua, *Iltis et al. 800* (WIS); Abancay, rd. from Abancay to Andahuaylas, banks of Río Pachachaca/Apurímac, near old bridge, *Weigend & Weigend 2000/315* (HUSA, M, NY, USM); Grau, Kairaneka, *Vargas 5827* (BSB, CUZ); Abancay, Matará, *Vargas 10008* (MO). **Arequipa:** Caravelí, Km 630 Panam. S, 14 km N of Puerto Chala, *Iltis et al. s.n.* (WIS); Caravelí, Quebrada Atico, *FLSP 1303* (MO); Caravelí, Km 621 Panam. S, 20 km N of Puerto Chala, *Iltis et al. 1570* (WIS); Caravelí, Atico S of Chala, *Tovar 2677* (MO, MSB, USM); Lomas de Atico, *Ferreyra 8888* (MO); Caravelí, Km 586 Panam. S, Lomas 6 km N of Atiquipa turnoff, *Weigend & Förther 97/718* (F, MSB, NY, USM); Arequipa, *Stafford D7* (K); above Arequipa, *Ferreyra 14284* (MSB, USM); Arequipa, slope toward Estanquillo Ahogado Chachani, *Arenas 87* (USM); 4 km N of Arequipa, Chilinas, hills on SW side of Río Chilinas, *Solomon 2788* (USM); Arequipa, Tiabaya, *Vargas 9437* (BSB, CUZ); Arequipa, Tingo, *Vargas 7906* (BSB, CUZ); Sánchez Cerro, *Cáceres et al. 3011* (BSB, HUSA); Islay, Lomas of Mollendo, ca. 10 km NW of Islay, *Dillon & Dillon 3956* (F, HUT, USM). **Cajamarca:** Cajabamba, rd. from Cajabamba to Cajamarca, 23 km from Cajabamba where the rd. drops to San Marcos, *Weigend et al. 97/315* (F, HUT, K, MO, MSB, NY, US, USM, W); Jaén, rd. from Jaén to San Ignacio, 68 km from Jaén, *Weigend et al. 98/470* (CPUN, F, M, USM); btw. Jaén & Puente Corral Quemado, *Ferreyra & Sánchez 19671* (USM); btw. Sallique and La Unión, *Campos et al. 9917* (MO); Río Huancabamba & Río Chotano confluence, rd. Olmos to Río Marañón, *López et al 4097* (HUT); 2 km W of Cajamarca, *Hudson 1097* (MO); Celendin, Llanguat, *Mostacero 964* (MO); Santa Cruz, 31 km from Santa Cruz on rd. to Catache, 3 km E of Catache, *Weigend et al. 7543* (BSB, HUT, M, USM); Santa Cruz, 2 km from Santa Cruz on rd. to Catache, *Weigend et al. 7534* (BSB, HUT, M, USM); Chota, Chota–Huambos rd., 26 km from Chota, *Weigend & Dostert* 98/145 (CPUN, F, M, USM); Chota, Chota–Huambos rd., 9 km from Chota, directly E of Lajas, *Weigend & Dostert 98/142* (BSB, HUT, M, USM); Cajamarca, 1 km S of Jesús, *Sánchez & Sánchez 7189* (CPUN, F); 3 km on rd. from Bambamarca to Paccha, near Chala, *Weigend & Dostert 98/111* (CPUN, F, M, NY, USM). **Cuzco:** Urubamba, btw. Chincheros & Urquijos, *King et al. 239* (K, MO, NY, USM); rd. from Urubamba to Puna Huanoa, *Ackermann & Kollehn 265* (BSB, HUSA, M, USM); Ollantaytambo, Pachar, *Huamantupa et al. 3632* (BSB, MO); Ollantaytambo, *Schwabe s.n.* (B); Urubamba Valley, *Salinas 304* (BSB), *Herrera 1529* (MO); Urubamba Valley, Yucay, *Herrera 1366* (BM); Yucay, *Soukup s.n.* (USM *#20608*); near town of Urubamba, *Gade s.n.* (WIS); Huayoccari to Yanacocha, NW of Cuzco, *Nuñez 7096* (MO); Pomatales, 52 km from Cuzco on railway to Quillabamba, *Nuñez 7285* (MO); Urubamba, beyond the river, *Müller & Gutte 9435b* (LPB); Calca, rd. from Yucay to Calca, *Weigend & Weigend 2000/171* (HUSA, M, NY, USM); Calca, Calca, *Vargas 964* (BSB, CUZ); Calca, 4 km from Pisac on rd. to Calca, *Hawkes et al. 5148* (MO); Urubamba, rd. from Urubamba to Ollantaytambo, *Weigend & Weigend 2000/209* (HUSA, M, NY, USM); Chincheros, quebrada btw. Chinchero & Urquillos, *King et al. 239* (USM); Anta, rd. from Curahuasi to Cuzco, before bridge over Apurímac river, *Weigend & Weigend 2000/395* (HUSA, M, NY, USM); Limatambo, Cunyac, *Galiano et al. 5042* (BSB, MO); Yucay, *Soukup 10604* (USM); Convención, Quellomayo, *West 8017* (MO); Urubamba, Distr. Huayllabamba, *Tupayachi 927* (MO); Urubamba, Distr. Huayllabamba, *Tupayachi 126* (MO). **Huancavelica:** Huaytará, Pisco–Ayacucho rd., *Weigend & Förther 97/595* (F, MSB, USM); Huancavélica, Mejorada, btw. Izcuhaca & Acoria, *Tovar 1017* (USM); Huallhuas, near Mejorada, *Tovar 313* (USM); Huancavélica, rd. from Huancavélica to Palca, btw. Palca & Izcuchaca, *Weigend & Skrabal 5861* (BSB, HUT, M, USM); Huancavélica, 5 km E of Mejorada, *Stork & Horton 10911* (MO). **Junín:** Huancayo, betw. Huancayo & Mejorada, *Hjerting & Petersen 1384* (USM). **La Libertad:** Huamachuco, Hac. Cochabamba, *López 1441* (MSB, USM); Hac. Cochabamba, *Lopez & Sagástegui s.n.* (HUT 2789); Otuzco, above Río Pollo, *Rodriguez et al. 2239* (HUT, M, USM), *Rodriguez et al. 2236* (HUT, M, USM), *Rodriguez et al. 2237* (HUT, M, USM); localidad Nambuque, above La Cuesta, *Rodríguez et al. 2248* (HAO, HUT); Cerro Chologday, *Binder et al. 1999/198* (HUT); above Río Pollo, *Binder et al. 1999/211* (HUT, M, USM), *Binder et al. 1999/212* (HUT, M, USM); Samne, *López s.n.* (HUT 987); Samne, *Angulo s.n.* (HUT 1424); Membrillar, Otuzco, *López s.n.* (HUT 1530), *Ridoutt s.n.* (HUT 354); Membrillar–Llaguén, *López s.n.* (HUT 1439); Pataz, btw. Buldibuyo & Huylillas, *Sagástegui et al. 17327* (BSB, HUT); Pataz, *Sagástegui et al. 17283* (B); Buldibuyo to Tayabamba, *Weigend & Schwarzer 8008* (BSB, HUT, USM); Alpamarca near Retamas, *Sagastegui et al. 17283* (BSB, HUT); Trujillo, Cerro Chiputur, *Angulo & López s.n.* (HUT 1186), *López & Sagástegui 9165* (HUT, MO), *Sagástegui & Mostacero 11041* (HUT), *Mostacero et al. 766* (HUT); Cerro Campana, *Angulo s.n.* (HUT 2020), *Angulo & López s.n.* (HUT 715); Cerro Cabezón, *Mostacero et al. 766* (F, HUT), *Mostacero et al. 1482* (HUT), *Sagástegui & López 11021* (HUT), *Sagástegui & Mostacero 10472* (HUT); Simbal, *Mostacero et al. 715* (HUT); Laredo to Samne, *Angulo s.n.* (HUT 1314). **Lambayeque:** Chiclayo, Cerros de Tinajones, near Chonggoyape, *Cerrate de Ferreyra 5257* (MSB, USM); Sapotal (Motupe), *Llatas 746* (HUT); Ferreñafe, rd. from Batan Grande to Incahuasi resp. Ullurpampa (syn. Uyurpampa), just beyond Laquipampa toward Incahuasi, *Weigend et al. 8539* (B, HUT, USM); Sapotal near Motupe, *Llatas-Quiroz 746* (MO). **Lima:** Cajatambo, above Churín, *Ferreyra 3514* (USM); Chancay, near Trapiche, *Ferreyra 19146* (MSB, USM); Huamantanga, *Barclay 2310* (BM); Canta, S base of Huachoc Pass, 20 km N of Trapiche, *Hutchison 1025* (G, K, USM); Purrochuco, *Mathews 596* (E, G, K); Lima, btw. Trapiche & Huaral, *Ferreyra 11766* (USM); Huarochiri, below Langa, *Ferreyra 19066* (USM); Yauyos, rd. from Yauyos to Jauja, after Magdalena, *Weigend et al. 7240* (BSB, HUT, M, USM). **Moquegua:** Mariscal Cáceres, Torata, Quele, *Arakaki 281* (USM); 14 km E of Moquegua on rd. to Torata, *Weigend & Förther 97/847* (F, M, NY, USM); Quebrada de Torata, *Nuñez 13* (USM); Sánchez Cerro, Río Amarillo, *Cáceres 3010* (BSB, HUSA); Torata, *Hufford & McMahon 3836* (USM); Sánchez Cerro, Omate, *F. Cáceres H. 3237* (BSB, HUSA); rd. Omate to Arequipa, *Weigend & Schwarzer 7872b* (BSB, HUT, USM). **Piura:** Huancabamba, rd. betw. Huancabamba & Sondor, *Weigend & Dostert 98/202* (CPUN, F, M, USM); Huancabamba, btw. Huancabamba & La Cabeza, *Yarupaitán 1406* (USM). **San Martín:** Distr. Huallaga, valley of Río Abiseo,

30 km above Jucusbamba, *Hamilton & Holligan* 2013 (K). **Tacna:** Tarata, 78 km from Candarave on rd. to Tarata, 211 km W of Ilave, *Weigend & Förther 97/798* (F, MSB, NY, USM). VENEZUELA. **Falcón**: Bolívar, limestone ridges, *van der Werff & Wingfield 7465* (BSB, MO). **Mérida:** Ejido to San Juan de Acequias, *Tillett et al. 939-20* (MYF). **Monagas:** Btw. El Guácharo & La Cueva del Guácharo, *Bunting 2654* (MO). **Trujillo:** Betw. Timotes & Valera, *Oberwinkler 14283* (M); hwy. Valera to Timotes, 1 km SW of jct. to La Mesa rd., *Steyermark & Manara 125358* (M).

7.4. Mentzelia scabra Kunth subsp. **cordobensis** (Urb. & Gilg ex Kurtz) Weigend, comb. et stat. nov. Basionym: *Mentzelia cordobensis* Urb. & Gilg ex Kurtz, Revista Mus. La Plata, Secc. Paleontol. 5: 289. 1893. TYPE: Argentina. Córdoba: Totoral, Sierra Chica de Córdoba, close to Ascochinga, *P. G. Lorentz 265* (lectotype, designated here, GOET!; isotypes, BA not seen, K not seen). Figures 14, 15A, B.

Subshrub 0.6–0.8 m tall, stems 5–10? mm thick basally, with pale, strongly exfoliating bark. Leaves subsessile or shortly petiolate (lower leaves with petioles 5–7 mm), lamina (40–)55–70 × 20–40 mm, undivided or with one indistinct, round lobe on each side proximally, margin deeply or shallowly, irregularly serrate to lobulate; base truncate to cuneate; apex acuminate. Inflorescences dense; internodes 0.5–1(–2)× as long as bracts; pedicels 1–4 mm. Flowers with calyx tube with scabrid and glochidiate trichomes 0.1–0.6 mm long, longer trichomes both glochidiate and scabrid; calyx lobes 8–12 × 2 mm; petals (10–)12–15 × 10 mm; anthers ca. 60, outer ones markedly longer, ca. 10 mm, inner ones 5–6 mm. Capsule obovoid from conical base, 9–12 × 3–5 mm, covered with both scabrid (to 500 µm) and glochidiate (to 400 µm) trichomes.

Several extant potential type specimens exist: additional specimens cited with the protologue are *G. Hieronymus s.n.* and *O. Kuntze 7263*, both of which were apparently housed in Berlin and are now lost. Sleumer (1955: 418) referred to the Berlin specimen of *P. G. Lorentz 265* as type (F-10123, photo), which he had seen before the fire in Berlin in March 1943 destroyed it (Hiepko, 1987). Lectotypification is required, and the specimen from GOET is therefore selected here. *Mentzelia scabra* subsp. *cordobensis* is morphologically similar to *M. scabra* subsp. *chilensis*, its northern neighbor. However, the flowers (especially the petals) are smaller, the inflorescences are very dense, and the leaves are relatively large, membranaceous, and shallowly lobed. The precise geographical limits between the two subspecies are still poorly understood, but the southernmost representative specimen of *M. scabra* subsp. *chilensis* I have seen is from the department Tarija in Bolivia. Whether subspecies *cordobensis* is really absent from Bolivia is still unclear. According to the material seen so far, the subspecies is restricted to northwestern Argentina at elevations of 500–1800(–2600) m.

Additional specimens examined. ARGENTINA. **Córdoba:** Totoral, Casa Bamba, Sierra Chica de Córdoba, *Stuckert 3679* (G); Casa Bamba to El Dique San Roque, Quebrada del Río Primero, *Tirel 293* (G), *Hunziker 7007* (G); Colon, Sierra Chica, *Lossen 163* (M, MO), *Lossen 293* (M); Calamuchita, Dique de los Molinos, *Kraporickas & Cristóbal 14708* (WIS); Colon, Sierra Chica, 5 km W of La Calera, *J. & A. Solomon 4067* (MO); Colon, Salsipuedes, *Hunziker 1466* (MO). **Jujuy:** Dpto. Tumbaya, below Tilcará, btw. Volcán & Puente de León, *Beck & Paniagua 26668* (BSB, LPB); Santa Bárbara, *Deginani et al. 905* (MO). **Salta:** Viña, Quebrada de las Conchas, btw. Salamanca & El Hongo, rte. 68, Km 77–78.5, *Novara & Bruno 9615* (M); Dpto. Rosario de Lerma, Quebrada del Toro, rte. 51, Km 32.7, 7 km W of Quijano, *Novara 7521* (M), *Novara 6471* (Z); Rosario de Lerma, Quebrada del Toro, *Sleumer 2000* (B); Guachipas, Quebrada del Cebillar, 9.2 km SE from La Viña, *Krapovizkas & Schinini 30619* (G). **Tucumán:** Burruyacu, Nogalito, Quebrada de las Asperezas near Los Pinos, *Vervoorst 6785* (BR, W).

7.5. Mentzelia scabra Kunth subsp. **grandiflora** (G. Don) Weigend, comb. et stat. nov. Basionym: *Mentzelia grandiflora* G. Don, Gen. Hist. 3: 66. 1834. TYPE: Tab. 451 in Ruiz & Pavón, Anales Inst. Bot. Cavanilles 16 [Fl. Peruv. 5]: 424. 1958. Figure 15C, D.

Mentzelia cordifolia Dombey ex Urb. & Gilg, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 76: 74. 1900. Syn. nov. TYPE: [Peru. Lima:] *J. Dombey 747* (lectotype, designated here, P!; isotypes, B†, G!).

Subshrub ca. 1 m tall; stems 5–9 mm thick basally, with pale exfoliating bark. Leaves subsessile to shortly petiolate (lower leaves with petioles 3–7 mm), 30–50 × 25–30 mm, irregularly lobulate, margin irregularly serrate to mucronate; base subcordate; apex acuminate. Inflorescences dense; internodes ca. 1.5–3× as long as subtending bracts; pedicels 1–2 mm. Flowers with calyx tube with a mixture of glochidiate and scabrid hairs 0.1–1 mm long, longest trichomes mostly glochidiate; calyx lobes ca. 10 × 3 mm; petals 18–25 × 8–14 mm; stamens ca. 60; 10 outer ones markedly longer, ca. 13 mm, inner ones 5–6 mm. Capsule ovoidal with conical base, ca. 8 × 5–6 mm, covered with scabrid and glochidiate trichomes, only few scabrid trichomes (700–800 µm), all other trichomes glochidiate (200–800 µm).

This subspecies of *Mentzelia scabra* was the second species to be described in the group in South America, and its correct name has been completely overlooked so far. In herbaria (and Brako & Zarucchi, 1993), it is generally called *M. cordifolia* Dombey ex

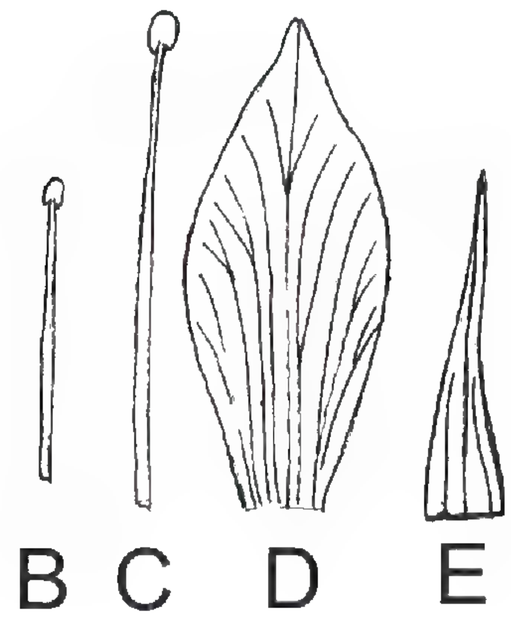


Figure 14. *Mentzelia scabra* subsp. *cordobensis*. —A. Flowering branch. —B. Inner stamen. —C. Outer stamen. —D. Petal. —E. Sepal. Drawn from *Novara & Bruno 9615*.

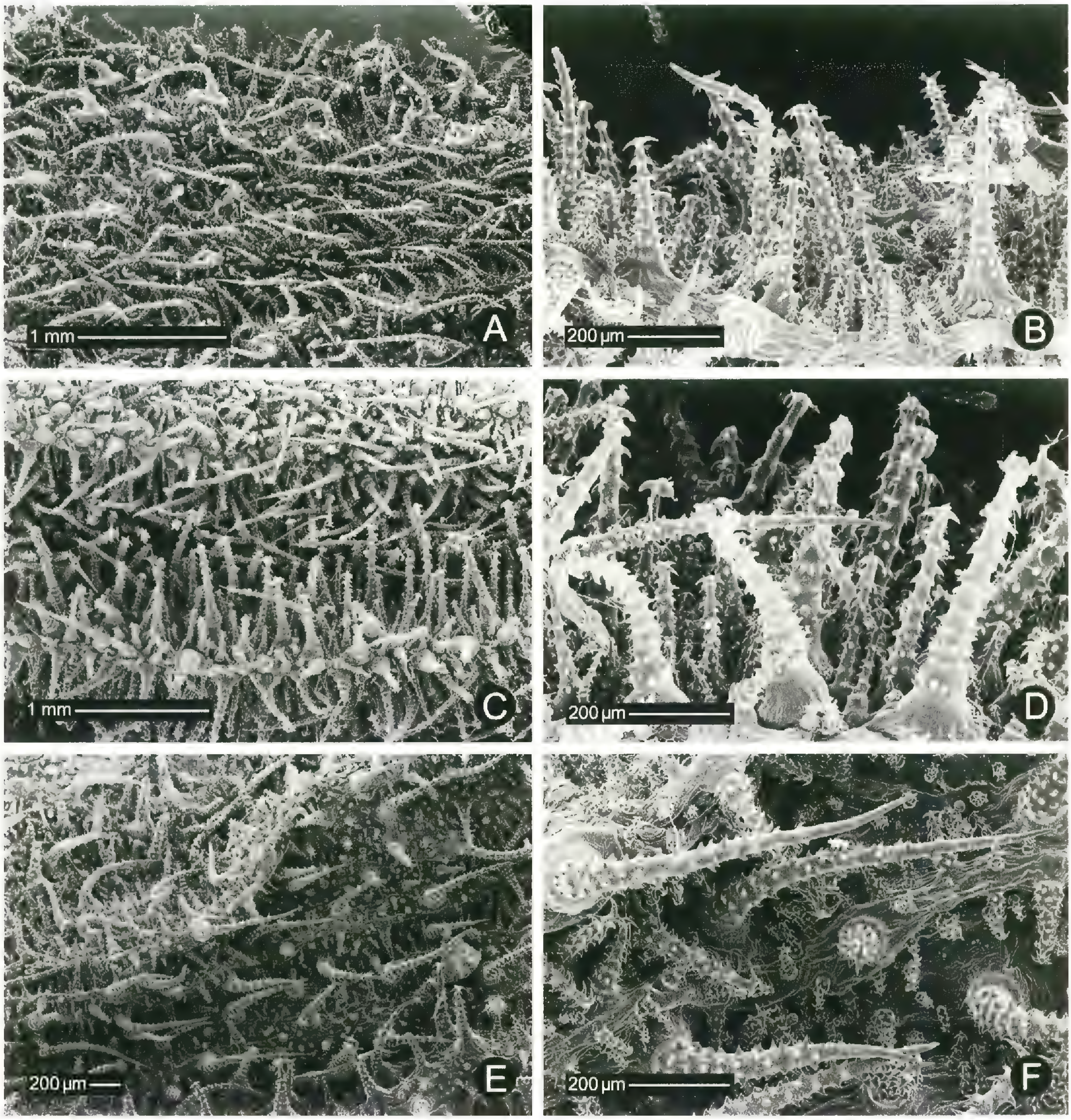


Figure 15. *Mentzelia scabra* subsp. *cordobensis*, subsp. *grandiflora*, and subsp. *scabra* indumentum on ovary. A–B. *M. scabra* subsp. *cordobensis*. —A. Overview, mixed indumentum with predominantly glochidiate trichomes (*Novara & Bruno 9615*). —B. Close-up showing a few scabrid trichomes (*Krapovickas & Cristóbal 14708*). C–D. *M. scabra* subsp. *grandiflora* (*Beigmann s.n.*). —C. Overview, mixed indumentum of mostly glochidiate trichomes. —D. Close-up of mostly glochidiate trichomes. E–F. *M. scabra* subsp. *scabra* (*Ramírez 1917*). —E. Overview, mixed indumentum of long scabrid and shorter glochidiate trichomes. —F. Close-up of glochidiate and scabrid trichomes.

Urb. & Gilg (or erroneously *M. cordifolia* Dombey), and that name has been indiscriminately used for nearly all suffrutescent *Mentzelia* species in Peru. *Mentzelia grandiflora* G. Don is the oldest name for that taxon, and it was directly based on the then-unpublished plate produced for *M. fulva* Ruiz & Pav. (Ruiz & Pavón, 1958: 424) for the *Flora Peruviae et Chilensis*. Don is thus the sole author of the species (not *M. grandiflora* Ruiz & Pav. ex G. Don, since George Don did not use the specific epithet "*fulva*"). The type collections of *M. fulva* and *M. cordifolia* are both from the Lomas de Chancay, where they were collected jointly by Dombey, Ruiz, and Pavón y Jiménez. There can therefore be no doubt about the synonymy of these three names. The names have been interpreted differently in the past, and Darlington (1934) mistakenly refers the material relegated here to *M. angurate* to *M. cordifolia* (e.g., *J. F. Macbride & W. Featherstone 130*, *J. F. Macbride 3215*, *J. McLean s.n.*, *F. W. Pennell 14472*). Due to the presence of several extant potential type specimens, lectotypification for *M. cordifolia* is required. It is here lectotypified on the

Figure 16. *Mentzelia sericea* Weigend. —A. Flowering branch. —B. Fruit. —C. Petal. —D. Sepal. —E. Outer stamen. —F. Inner stamen. From *Dostert 98/137*.

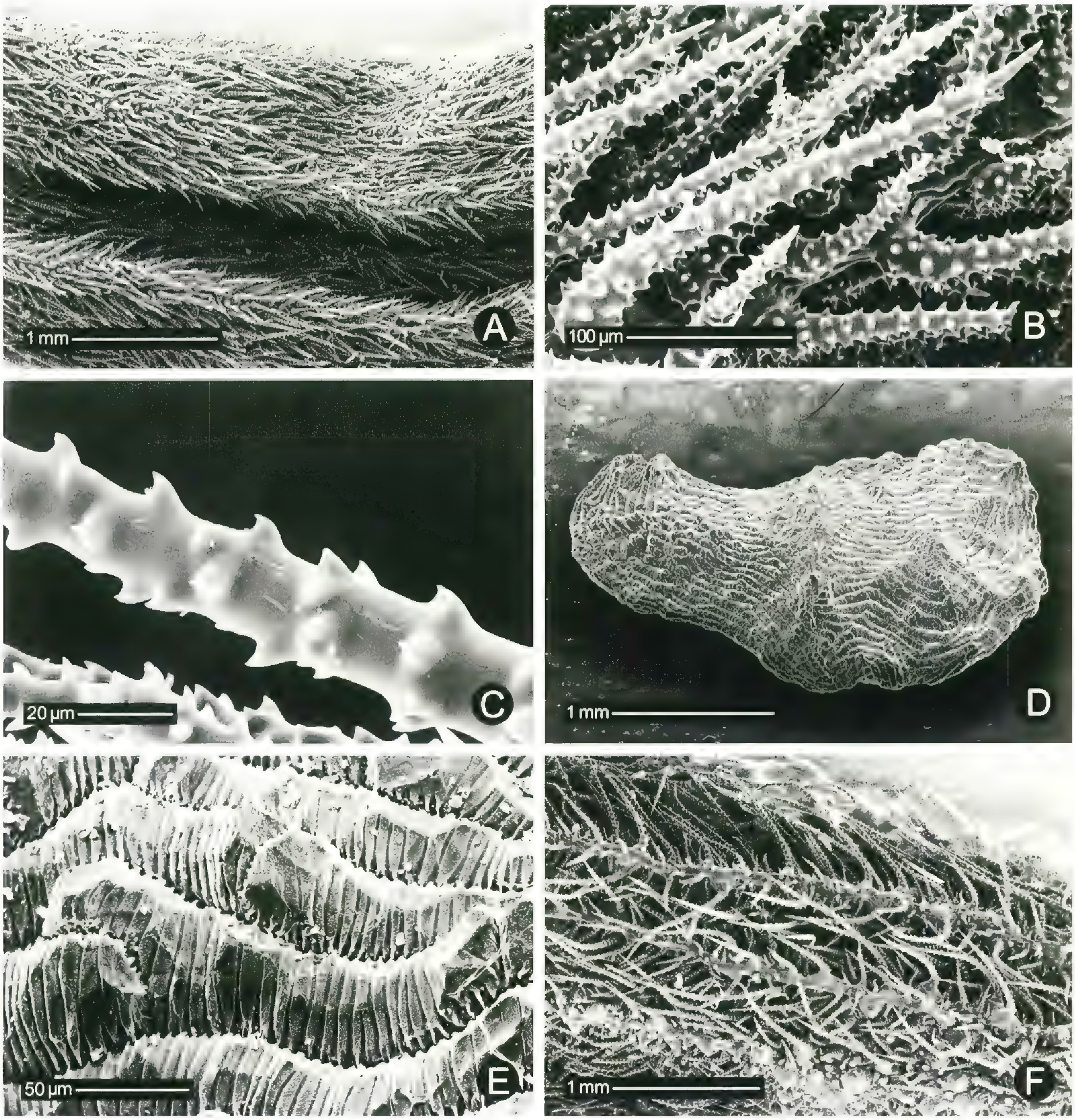


Figure 17. *Mentzelia sericea* Weigend and *M. sericea* × *M. scabra* subsp. *chilensis*, indumentum on ovary and seed. A–C. *Mentzelia sericea*, indumentum on ovary. —A. Overview, more or less homogeneous indument of short scabrid trichomes (*Ferreyra 19914*). —B. Close-up of densely overlapping acroscopic scabrid trichomes (*Weigend et al. 7468*). —C. Median portion of scabrid trichome (*Ferreyra 19914*). D, E. *Mentzelia sericea*, seeds (*Dostert 98/137*). —D. Overview, note the irregularly angular shape. —E. Detail of testa, note the indistinct transverse striations and slightly thickened ends of the cells. F. Putative natural hybrid of *M. scabra* subsp. *chilensis* × *M. sericea* (*Weigend et al. 98/527*), indumentum on ovary, showing the predominance of acroscopic, scabrid trichomes (as in *M. sericea*), but they are relatively widely spaced and interspersed with scabrid and glochidiate trichomes (as in *M. scabra* subsp. *chilensis*).

specimen at P, which was seen and annotated by Urban and Gilg (1900).

Mentzelia scabra subsp. *grandiflora* is relatively poorly documented, but we fortunately managed to re-collect the taxon in 2002, which contributed much to our understanding of the subspecies. It is primarily defined by the combination of relatively large leaves and flowers, and by the predominance of glochidiate trichomes on its ovaries, but otherwise is very similar to *M. scabra* subsp. *chilensis*.

Distribution and habitat. *Mentzelia scabra* subsp. *grandiflora* grows at the lowest elevations, by far, of any subspecies of *M. scabra* and is the only form that is apparently found in the wet lower part of the seasonal coastal lomas formation in Peru. In lomas where both *M. scabra* subsp. *chilensis* and *M. scabra* subsp *grandiflora* are found, the former is restricted to the upper limit of the lomas formation (800–1200 m) in sandy soils, whereas the latter is found between rocks at elevations below 400 m.

Additional specimens examined. PERU. **Arequipa:** Islay, Lomas de Mollendo, 10 km NW of Islay, *Dillon & Dillon 3956* (USM). **Lima:** Lima, Lomas de Amancaes, *Pavón s.n.* (G); Lomas de Manchay, Valle de Lurín, *Beigman s.n.* (USM); Lomas de Manchay, *Ferreyra 9585* (USM); Prov. Cañete, Lomas de Asia, *Ridout s.n.* (USM #13032); Chancay, Lomas de Chancay, *Ferreyra 8677* (M, USM); Yauyos, rd. from Quilmaná to Km 122 of Panamericana Sur, Lomas de Quilmaná, *Weigend et al. 7348* (BSB, USM).

7.6. Mentzelia scabra subsp. **scabra** Kunth. Figure 15E, F.

Strongly branched shrub 0.8–2 m tall; stems 10–20 mm thick basally, bark dark brown to grey with pale lenticels, not exfoliating. Leaves subsessile to shortly petiolate (lower leaves with petioles up to 20 mm); lamina widely ovate, 55–150 × 35–70 mm, margin irregularly serrate to lobed, lobes short (incision less than 1/3 of distance to midvein). Inflorescence dense; internodes shorter than or as long as bracts; pedicels 2–3 mm. Flowers with calyx tube densely set with scabrid and glochidiate hairs 0.1–0.7 mm long, the longest trichomes mostly glochidiate; calyx lobes 8–10 × 2 mm; petals 25–30 × 14–16 mm; stamens (80 to)100 to 120, outer ones ca. 18 mm, inner ones 10–13 mm. Capsule subsessile, obovoid, 10–14 × 5 mm, covered with scabrid trichomes ca. 600–800 µm long with a long smooth apex and glochidiate trichomes ca. 200–300 µm long.

Mentzelia scabra subsp. *scabra* appears to be rather narrowly endemic to southern Colombia, with all collections coming from more or less the same place. *Mentzelia scabra* subsp. *scabra* is geographically intercalated between the Venezuelan and Ecuadorean populations of *M. scabra* subsp. *chilensis*, but differs from that subspecies by the shorter internodes in the inflorescence, shrubby habit with persistent bark, wider petals, more numerous anthers, and larger leaves. This taxon is more poorly defined than the other subspecies, and better and more complete collections are clearly desirable. Its closest affinities are with *M. scabra* subsp. *chilensis*, which differs by having smaller flowers with fewer stamens and/or by its subshrubby habit. This subspecies is found in relatively moist situations in Andean scrub communities in Colombia at elevations of ca. 2000 m.

Additional specimens examined. COLOMBIA. **Nariño:** Mpio. Taminango, 58 km from Pasto to Popayán, near El Tablón, *Ramírez 1917* (MSB, PSO); btw. Túquerres & Pasto, *Triana s.n.* (G, P), *Triana 5111* (COL); valley of Guaitará, *Weigend & Ramírez s.n.* (COL, M, PSO), *López-Palacios & Idrobo 3778* (COL); Yacanquer, *Fábrega 520* (COL); Imués, Km 2 W of El Pedregal, *Gentry et al. 64397* (PSO); Imués, Corregimiento El Pedregal, bridge of Guáitara, *de Benavides 6426* (PSO); San José, bridge over Río Juanambú, *de Benavides 5242* (PSO); Taminango, Km 58 Pasto–Popayán, near Tablón, *Ramírez 1917* (PSO); Tangua, Vereda Tapialquer Bajo, *Ramírez 5312* (PSO); Yacuanquer, Vereda de Tasnaque, *Ramírez 614* (PSO). **Province unknown:** *Humboldt & Bonpland s.n.* (P); San Pablo, *Humboldt & Bonpland 3520* (P).

8. Mentzelia sericea Weigend, sp. nov. TYPE: Peru. Cajamarca: Contumazá, rd. Contumazá to Chilete, 2500 m, *M. Weigend, N. Dostert & K. Drießle 97/452* (holotype, USM!; isotypes, CPUN!, F!, M!). Figures 16, 17A–E.

Haec species *Mentzeliae scabrae* affinis, sed ab ea ovario trichomatibus scabridis, albis, appressis, usque ad 0.5 mm longis instructo differt.

Shrub 0.6–0.8 m tall, stiffly erect; stems basally 5–15 mm thick, lignescent, extremely brittle, with pale, strongly exfoliating bark, young shoots densely covered with glochidiate and scabrid trichomes. Leaves petiolate, petioles 2–15 mm, upper leaves subsessile; lamina ovate, 40–75 × 20–55 mm, shallowly lobed or only irregularly mucronate or serrate; base subcordate to subcuneate; apex acuminate. Inflorescence frondose, lax, widely branched (branching angle between paraclades of dichasia less than 90°); internodes 2–5× as long as subtending bracts (up to 15 cm); bracts 10–35 × 5–20 mm; pedicels 1–3 mm. Flowers with calyx tube conical, ca. 5 × 2 mm, densely covered with appressed scabrid trichomes; calyx lobes 11–12 × 3 mm, long-acuminate from narrowly ovate base; petals widely obovate, 20 × 15 mm, base narrowed, apex apiculate, golden yellow to pale orange; stamens 70 to 90, 10 outer ones markedly longer, ca. 18 mm, inner ones only 10–13 mm, anthers ca. 1 × 0.75 mm. Capsule clavate, 10–14 × 5 mm, densely covered with appressed, scabrid trichomes 300–500 µm long, lustrous, white.

Mentzelia sericea has a very characteristic indumentum of appressed scabrid trichomes on both ovary and fruit and is thus immediately recognizable. *Mentzelia angurate*, which also has only scabrid trichomes, is easily distinguished by its many trichomes to 1.5 mm long, globose (vs. clavate) capsules, a yellow, erect (vs. white, appressed) indumentum, and decumbent (vs. erect) habit. It remains unclear whether *M. sericea* is more closely allied to *M. angurate* or *M. scabra*, but it is more similar in habit to the latter (stiffly erect, not decumbent as *M. angurate*), and the loss of one trichome type may well be homoplastic. Phylogenetic analyses will be required to clarify its affinities.

Distribution and habitat. Mentzelia sericea is apparently endemic to northern Peru, and all collec-

tions known so far come from the neighboring departments Lambayeque, La Libertad, and Cajamarca. It is restricted here to the Pacific slope of the Andes and is replaced by *M. scabra* subsp. *chilensis* in all the inner-Andean valleys. There are a few intermediate specimens that have an anisotrichous indument of scabrid hairs that are mostly 1 mm long and interspersed with some shorter trichomes. The trichomes are also not as strictly appressed as is typical of *M. sericea*. These specimens (*Soriano & Guevara 436*, *Sánchez 3849*, both F) seem to represent hybrids with *M. scabra* subsp. *chilensis*. These plants were found relatively far inland in the Río Jequetepeque valley, where the contact zone between the two taxa would be expected.

Mentzelia sericea is found in dry scrub communities, on scree slopes, and in rock falls at elevations of 350–2500 m in northern Peru.

Paratypes. PERU. **Cajamarca:** San Miguel, rd. Chilete to San Miguel, *M. Weigend, T. Henning & O. Mohr. 7468* (BSB, HUT, M, USM); btw. Platanar & Lives (carretera hacia el pueblo Unión Agua Blanca), *E. Rodríguez R. et al. 2437* (HUT); Cajamarca, rd. to Pacasmayo, 48 km from Cajamarca, *M. Weigend, Th. Franke & J. Skrabal. 98/530* (CPUN, F, M, USM); 1 km S of Jesús, S of Cajamarca, *I. Sánchez V. 7189* (F); 1 km above San Juan, *I. Sánchez V. 3849* (F); rd. from Cajamarca to Chilete, Km 70, *N. Dostert 98/137* (CPUN, F, M, USM); rd. from Chilete to San Bernardino, above Pichana, *J. Cabanillas & J. Guevara B. 499* (F); Paredones, rd. Chilete to San Pablo, *A. Sagástegui A. 7914* (HUT, WIS); Asunción–San José, *Sagástegui et al. 10157* (HUT, MO); Contumazá, near Cascas on rd. from Cascas to Trujillo, *M. Weigend, T. Henning & O. Mohr 7650* (BSB, HUT, M, USM); 83 km from Cajamarca on rd. to Pacasmayo, *M. Weigend, N. Dostert & K. Drießle 97/432* (F, HUT, M, NY, USM); Km 85.5 on rd. Tembladera to Chilete, *S. Baldeón 852* (USM); El Balconcito below San Benito, *A. Sagástegui A. 16426* (BSB, HAO); La Herilla (Guzmango), *A. Sagástegui A. et al. 9698* (HUT, MO); San Antonio–Portachuelo (Carretera Ascope–San Benito), *L. López & A. Sagástegui A. 7921* (HUT); Los Chirimoyos (San Benito–Guzmango), *A. Sagástegui A. & L. López 10526* (HUT, MO); Cuesta del Espino (San Benito–Hda. San Lorenzo), *Sagástegui 3749* (HUT); Cushtón (Contumazá–Chilete), *L. Mostacero et al. 3377* (HUT); San Benito, Las Chirimoyas, Guzmango, *A. Sagástegui A. & S. Leiva 15535* (F, HAO, M); rd. Contumazá to Chilete, *M. Weigend, N. Dostert & K. Drießle 97/452* (F, HUT, M, USM); below Cascas, *R. Ferreyra 19914* (MSB, USM); alrededores Cerro Cuculicote, *L. López et al. 9264* (HUT); San Pablo, Sangal, *A. Sagástegui A. 15617* (F, HAO, M); San Miguel, *A. Weberbauer 3923* (G); Chota, Llama, rd. Llama to Chiclayo, 15 km from Llama, *M. Weigend & N. Dostert 98/162* (CPUN, F, M, USM). **La Libertad:** Otuzco, Samne at the Otuzco–Huamachuco turnoff, *R. Ferreyra 7660* (M, MOL, USM); Samne, Otuzco, *L. López s.n.* (HUT 987); above Samne, btw. Trujillo & Otuzco, *R. Ferreyra 14083* (USM); Casmiche (Samne–Otuzco), *A. Sagástegui A. et al. 11494* (HUT, MO); Trujillo, Plazapampa, *H. de Cevasco s.n.* (M, MO, USM); Trujillo, Cerro Chiputur, *L. López 373* (USM); Prov. Gran Chimú, planta eléctrica to Tambo La Lima, *A. Sagástegui et al. 17098* (B, F). **Lambeyeque:** Prov. Olmos, 2 km E of Olmos, *A. Gentry 22590* (USM).

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NINE EASY STEPS FOR CONSTRUCTING RELIABLE TREES FROM PUBLISHED PHYLOGENETIC ANALYSES[1]

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ABSTRACT

Classification and biogeography should be informed by reliable phylogenetic reconstruction. This may be recovered from the mainly speculative literature by a nine-step protocol of critical methods, the "operative transform." Piecemeal total evidence is attained by combining separately published support values for the same branch arrangement with Bayes' Formula. Joint probability correction is advanced for multiple test problems of conclusions of monophyly necessarily regarded as sets. Tables are given for conversion of nonparametric bootstrap values and decay indexes into probabilities, based on four-taxon simulations. Uncontested morphology-based branch arrangements are assigned high priors. Probabilities associated with unaccounted assumptions are addressed by a general correction factor. A probabilistic calculation allows the inference of two or more reliable internodes from a series of less well-supported internodes. A patristic distance of at least two internodes is used to support inference from molecular data alone of an intermediate lineage for recognition of supraspecific segregate taxonomic entities. Recognizing paraphyletic taxa when appropriate preserves attributes of inferred functional evolution providing taxic unity and value. Evolutionary Lazarus taxa contravening Dollo's Rule against re-evolution of complex traits justify interpretations of reticulation. This method of evaluation of the decisiveness of published molecular studies is exemplified with previously published cladograms focused on Malpighiales and the moss family Pottiaceae. Additional consideration of evolutionary process not reflected in phylogenetic analysis leads to reliable hypotheses of evolutionary relationship.

Key words: Bayesian, binomial confidence interval, bootstrap, bryophyta, cladograms, decay index, Malpighiales, multiple tests, operative transform, paraphyly, reliability.

It is clear from a perusal of published papers on phylogenetics that the past two or three decades of excitement in clarifying ancestral relationships, first with morphological traits and now with molecular or genomic data, have fueled a largely speculative literature. Whether the authors are theorists or working systematists, an emphasis has been laid on statistical "power" in distinguishing lineages rather than vigorous avoidance of type I error that the null hypothesis of no or at least no distinguishable phylogenetic signal is false when in fact it is true. Confirmation of conclusions of monophyly is commonly left to others. Although nowadays, with ever-increasing data, support values for branch arrangements (BAs) can be very high or apparently statistically certain, many assumptions (e.g., regarding alternative sequence alignments) remain unaddressed or are only now being investigated, and the contribution of this additional uncertainty impacts the reliability of published results. For any hypothesis test, a third alternative is needed: the metahypothesis that there is insufficient information from all relevant factors to tell if the null is rejected or not.

This paper is written solely for the second-level users of published cladograms, such as systematists, biogeographers, ecologists, and population biologists, who require well-grounded theories of ancestral relationship on which further, contingent study may be based. Nine simple steps are presented here as a method of identifying reliable conclusions of monophyly (resolved BAs) and, beyond this, hypotheses of descent with modification from the published literature. Frequentist statistics are avoided, and somewhat easier probabilistic or Bayesian concepts are emphasized. The nine steps are for situations in which published papers are the only source of data, and we must then necessarily resort to some shortcuts and ad hoc solutions. It is out of the question, however, that the second-level user, commonly not a phylogeneticist, might simply redo the phylogenetic study and investigate any additional possible sources of uncertainty.

[1] This is the result of an investigation into the reliability of phylogenetic estimation spanning 10 years. Hard-won concepts are often "obvious" post hoc. Basic concepts were developed during exchanges with J. Lyons-Weiler and others on the listserver Taxacom, and elaborated in response to comments from P. M. Eckel, J. Felsenstein, B. Goffinet, J. Huelsenbeck, P. Lewis, B. Larget, R. E. Magill, and many others. Olaf Werner was gracious in sending me a Nexus file of his original data set. I take responsibility for the central ideas in this paper to the extent they are new. The solvent criticism of anonymous reviewers is much appreciated.

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"Reliability" is here defined in the Bayesian context as that level of confidence or credibility needed to confidently place a bet on being correct in light of what might be lost if wrong. Given that what is risked is not only phylogenetic science in advancing a possibly wrong conclusion of monophyly, but necessarily also the dependent science of second-level users, then high confidence is required. In this paper, a probability of 0.95 is accepted as minimum reliability, meaning that no more than one out of every 20 BAs is wrong (and which one is unknown). This level is standard for fields, such as experimental psychology, physiology, and ecology, that commonly involve statistical evaluation of test results, and is not arbitrary in that it establishes the expected across-the-board level of reliability of research in the whole field.

Materials and Methods

Software used in this study was MrBayes (Huelsenbeck & Ronquist, 2001) for Monte Carlo Markov chain (MCMC) Bayesian analysis; PAUP* (Swofford, 1998) for maximum parsimony analysis, nonparametric bootstrapping, and jackknifing; RANDSET (Zander, 1999) for generating totally contrived random sets of either binary digital or DNA data; and VassarStats (Lowry, 2005) for binomial probabilistic calculations.

Two published treatments are here used as examples for operative transformation into cladograms with all branch arrangements supported at 0.95 or greater, or collapsed. The Savolainen et al. (2000) maximum parsimony cladogram of the Malpighiales based on *rbcL* gene sequences, among other results, split the Euphorbiaceae sensu Webster into five separate families, these accompanied by certain morphological differences. Of these, Chase et al. (2000: 689) wrote: "Nearly all families are well supported as monophyletic..." The present work asks: "Are these families supported by molecular data alone as distinct from the traditional Euphorbiaceae?" A second study, by Werner et al. (2004), focused on the moss family Pottiaceae using *rps4* chloroplast gene sequences and Bayesian analysis. In this study of 73 species, 51 of Pottiaceae and 22 of species from related families, the Pottiaceae group (with *Scopelophila* (Mitt.) Lindb. as basal) was almost entirely terminal. One genus, *Timmiella* (De Not.) Limpr. in Rabenh., was segregated toward the base of the cladogram, while exemplars of two genera of other families, Ephemeraceae and Cinclidotaceae, were embedded in the Pottiaceae. The present study investigates the questions: "Is the Pottiaceae to lose *Timmiella*? Should Ephemeraceae and Cinclidotaceae be synonymized with Pottiaceae to avoid paraphyly of the Pottiaceae?" More generally, the present paper asks: "What reliable hypotheses of evolutionary relationship can be extracted from published cladograms?"

In short, continuing refinement of a method (Zander, 2001, 2004, 2006) resulted in an optimal nine-step procedure for evaluating the reliability of cladograms published in the literature. As this paper is methodological, the Results summarize the technique.

Results

This paper introduces several new or possibly unfamiliar concepts and acronyms, summarized here: **AB** represents a posteriori evolutionary support indicating shared ancestry of taxa A and B as measured by branch length (steps or state changes in maximum parsimony); (**AB**) is a lineage of two taxa of shared ancestry, in which A and B in this paper are always the terminal sister taxa or lineages of the optimal arrangement among three taxa A, B, and C; **BPP** is Bayesian posterior probability, calculated via the well-known Bayes' Formula or as given in the publication being analyzed; **PBA** is the probability of branch arrangement, and is here used as equivalent to the Bayesian credible interval and is a general term for both the BCI and BPP; **BP** is nonparametric bootstrap proportion; **JP** is jackknife proportion; **DI** is decay index or Bremer support; **BCI** is binomial credible interval, essentially one minus the chance by chance alone of support of AB steps or better out of all support for A or B in all NNI combinations of branches in a four-taxon data set; **NNI** is nearest neighbor interchange, or switching of each of two terminal lineages with the next lower branch, e.g., (AB)C, (AC)B, and (BC)A; **IRI** is implied reliable internode, a single reliable internode representing the pooled less-than-reliable support of two or more chained internodal branch arrangements later collapsed into one reliable internode; **FIRI** is the formula for calculating the probability of an implied reliable internode; α (alpha) in hypothesis testing is probability of a type I error or, in a nonparametric context (Kolaczkowski & Thornton, 2004), the chance of obtaining these data by chance alone, where BCI is the complement or one minus α.

Efforts at eliminating intolerable uncertainty long embedded in standard methodologies resulted in a nine-step "operative transformation" of published cladograms into trees with resolution restricted to conclusions of monophyly at 0.95 or greater probability of being correct. The chance of a BA being correct is represented here as the probability of branch arrangement (PBA), and refers to either the Bayesian posterior probability (BPP) or a nonparamet-

Table 1. Approximate maximum assignable fail-safe four-taxon binomial credible intervals (BCIs) calculated from the nonparametric bootstrap proportion (BP) for various branch lengths as the BCI. The probability of branch arrangements being correct (PBA) is approximated by the chance of at least one trait shared by the terminal pair representing joint ancestry, being the chance of n steps (the branch length) minus one step by chance alone out of the total pairs of shared traits among three non-root branches (i.e., AB + AC + BC), using exact binomial calculation at one third probability. Interpolate intermediate values as necessary.

	Approximate PBA (as BCI)				
BP	5 steps	10 steps	15 steps	20 steps	25 steps
1.00	0.99	0.99	0.99	0.99	0.99
0.95	0.95	0.97	0.98	0.98	0.98
0.90	0.93	0.95	0.95	0.95	0.95
0.85	0.90	0.90	0.92	0.92	0.93
0.80	0.85	0.88	0.90	0.90	0.90
0.75	0.83	0.83	0.85	0.86	0.87
0.70	0.79	0.78	0.81	0.80	0.82
0.65	0.70	0.75	0.78	0.77	0.78
0.60	0.66	0.70	0.74	0.75	0.76
0.55	0.62	0.64	0.65	0.71	0.72
0.50	0.57	0.60	0.61	0.64	0.65

ric bootstrap value matched with a probability of the branch arrangement being correct with four-taxon simulations and previously dubbed (Zander, 2001, 2004) the binomial confidence interval (BCI) or here renamed binomial credible interval to avoid mixing frequentist and Bayesian processes of evaluation.

STEP ONE

Change support measures to PBAs. Nonparametric bootstrap values are commonly given in published cladograms, but these are not generally taken as direct measures of probability. If the published study were redone using one of the newer bootstrap corrections, such as double-bootstrap or similar methods (e.g., Rodrigo, 1993; Zharkikh & Li, 1995; Efron et al., 1996; Sanderson & Wojciechowski, 2000; J. Farris in Salamin et al., 2002; Aris-Brosou, 2003; Susko, 2006), then this could be used as PBA. In lieu of redoing the study, however, an approximation table (Table 1) is given here for conversion of BP to BCI values (Zander, 2004). While the relationship between the BP and BCI is not linear, interpolation of intermediate BPs is not difficult.

Four-taxon data simulations at various branch lengths were contrived to give particular BPs. Given that convergence affects all possible pairings of the three non-root branches (a.k.a. nearest neighbor interchange), the chance of at least one trait from shared ancestry belonging to the terminal pair is the chance of n steps (the branch length of the terminal pair) minus one step occurring by chance alone out of the total pairs of shared traits among three non-root branches, using exact binomial calculation at one-third probability. Alternatively, it is the same as one minus the chance that all steps of the terminal pair occurred by chance alone among the total pairs of shared traits among the three non-root branches.

We here define A, B, C, etc., as representing lineages: (AB), (AC), and (BC), etc., as lineages hypothesized to have shared ancestry; and AB, AC, BC, etc., as the branch length (support in numbers of shared advanced traits) leading to those lineages. As example of conversion of BP to BCI, a BP of 0.85 is produced by lineages A and B sharing 10 traits, when AB = 10, AC = 5, and BC = 5. So of the 10 shared traits of (AB), about five are expected to be convergent, matching the five of (AC) and (BC), but this expected number has random variation and there is a certain chance that all 10 traits of (AB) are randomly generated (by convergence). With a null hypothesis of all data randomly generated, the chance of (AB) having nine or fewer out of 20 total synapomorphies (paired traits) at one third by chance alone is 0.91, which is the same as the chance of one true synapomorphy for (AB), the minimum number of synapomorphies needed for support of a hypothesis of shared ancestry for (AB)C.

Four taxa are sufficient for determining an effective approximation of the true probability of shared ancestry from the BP given in the literature even if the original BP was generated on the basis of the whole data set, not just that of the three terminal taxa. One does not know if the original BP was calculated largely on the basis of NNI or involves branch exchanges elsewhere in the cladogram, but NNI may

be assumed for purposes of approximation. "Privileged" branch arrangements are those segregated by the software as having much higher PBAs than expected from chance alone, and which are thus non-null cases from a null hypothesis of a star at the base of the cladogram and all variation random, similar to the "thinned counts" of Efron (2005). In fact, the BP is rarely over 0.50 from random data alone.

Both maximum parsimony and Bayesian procedures, when used with several small data sets generated by RANDSET (Zander, 1999) of ca. 20 taxa and 20 to 200 characters (no parsimony non-informative characters) of either 2- or 4-state random data, usually but not always resulted in fully resolved cladograms. Reliability measures of 0.01–0.50 BPP were obtained for the Bayesian analysis (with datatype = standard, 500,000 generations, burnin = 1000), and < 0.05–0.26 BP (heuristic search, with 2000 replications) for the parsimony analysis (BP values less than 0.50 may not be fully compatible with the tree of maximum parsimony). With larger random data sets, e.g., 20 or 73 taxa and 588 characters, BPs were commonly smaller, < 0.05–0.12, while BPPs were more variable, averaging about 0.20 BPP but rarely individually as high as 0.82. With large random data sets, branch lengths are generally between 100 and 200 steps, indicating that length of branches is no more indicative of accuracy than is degree of cladogram resolution.

One might then assume that in practice, all branch arrangements with BPPs and BPs less than 0.50, figures that are usually not given anyway, are indistinguishable from those generated by totally random data. On the other hand, all BPPs and BPs larger than 0.50 are amenable to probabilistic generation of single composite, reliable internodes via the IRI formula (Step Five) because they represent the equivalent of tosses of phylogenetically loaded dice.

Such BAs may be viewed as sufficient statistics (including all relevant information and, thus, independent) in the same way that uncontested groups based on descriptive data are considered phylogenetically isolated except for convergence with immediately alternate branch arrangements (a local star). Uncertainty is probably local and well approximated by the four-taxon tables for BAs with high BPs, which are the most critical branch arrangements. Given that the BCI approximates the likelihood of (AB)C,D being correct, it is also the local BPP, given uninformative priors and a normalizing constant of unity. Additional information and discussion are detailed in the Web Supplement (Zander, 2007). A similar local measure of branch arrangement reliability, the approximate likelihood-ratio test (Anisimova & Gascuel, 2006) is also based locally (the branch of interest and four adjacent branches) and apparently performs well in simulations with larger trees; it is, however, implemented by specialized software using the original data and cannot be used to easily interpret published cladograms.

In Figure 1, we examine a much modified version of the Malpighiales *rbcL* maximum parsimony cladogram of Savolainen et al. (2000) for the level of molecular support for splitting the Euphorbiaceae into five families. The bold lines show the tree supporting the split. From the original cladogram, branch lengths are given above the lines and BP below. With branch lengths and BP, the BCI can be estimated from Table 1, and because the BPs were all above 0.50 and thus not expected by chance alone, the BCI can be taken as PBA. The five-part partition comprises the pre-cladogram concept of Euphorbiaceae.

The four-taxon BCI equivalent of Bremer support or the decay index (DI) is easily calculated, but a table (Table 2) is given here for convenience. This is the "local DI" (Zander, 2004), with no provision for collapse of a clade due to influence of a distant lineage, but justifications given above for approximations using four-taxon simulations also apply. Like the BCI for the BP, it is equal to AB minus one step out of AB + AC + BC trials where AC and BC are equal (as possible), and AC and BC are each set equal to AB minus the DI in number of steps. Alternatively, it is equal to one minus the probability of AB out of all shared pairs of traits. Thus, for a branch length of 10 steps and a DI of 6, AB = 10 and AC and BC are assumed both equal to four as a maximum to be fail-safe by maximizing the possible contrary data, and the BCI is the chance of nine steps out of 18 at probability one third by chance alone.

Thus, when branch length and either BP or DI are given, the probability that the branch arrangement is correct may be approximated by reference to Table 1 or 2. To repeat, the BCI is an acceptable approximation of the BP or DI of the true PBA, in lieu of fully redoing the analysis with maximum parsimony and a technique like double bootstrapping, or with Bayesian MCMC.

When Bayesian posterior probabilities are given, they may be at least provisionally accepted directly as a support measure (PBA). There are certain papers (Suzuki et al., 2002; Zander, 2004; Lewis et al., 2005) that indicate that these values are too low, at least with short branches (but see Kolaczkowski & Thornton, 2006). Table 3 demonstrates with artificial four-taxon data sets that BPP is too low with short branches. Further study with longer branches indicates that BPP and BCI begin to approximate at about

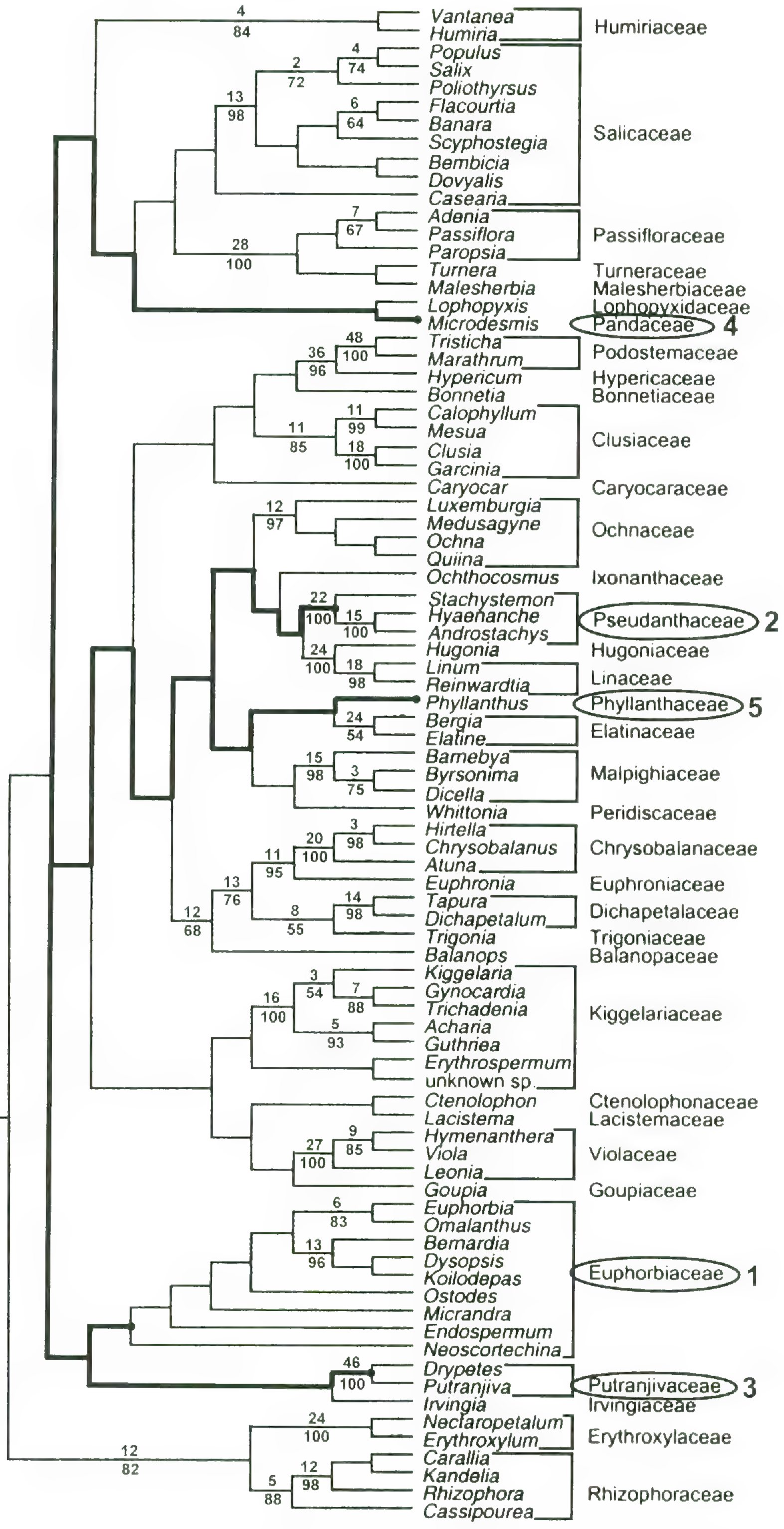

Figure 1. A single *rbcL* tree of Malpighiales from Savolainen et al. (2000) as presented by Chase et al. (2000). Euphorbiaceae sensu Webster is split (bold lines) into five families. These are numbered in order of importance for evaluation of multiple test problems. Branch length is above lines, nonparametric bootstrap proportion below. Reprinted with permission.

Table 2. Approximate maximum assignable fail-safe four-taxon PBAs calculated from the DI for various branch lengths. Interpolate intermediate values when necessary.

	Approximate PBA (as BCI)						
DI	3 steps	4 steps	5 steps	10 steps	15 steps	20 steps	25 steps
1	0.57	0.56	0.52	0.54	0.53	0.53	0.52
2	0.79	0.74	0.71	0.64	0.62	0.60	0.59
3	0.96	0.95	0.85	0.75	0.70	0.67	0.65
4	–	0.99	0.95	0.84	0.78	0.74	0.72
5	–	–	0.99	0.91	0.85	0.80	0.78
6	–	–	–	0.96	0.90	0.86	0.83
7	–	–	–	0.98	0.94	0.90	0.87
8	–	–	–	0.99	0.97	0.94	0.91
9	–	–	–	0.99	0.99	0.96	0.94
10	–	–	–	0.99	0.99	0.98	0.96

30 steps. Because the window of reliability is only 0.05, a slight difference matters greatly.

STEP TWO

Begin evaluation of published conclusions of monophyly. Partition the cladogram into phylogenetically isolated groups, using pre-cladogram concepts of classification or relationship, as best possible. Multiple test (multiple comparison) problems are a major fault with phylogenetic analysis as presented in the literature. Any conclusion of monophyly is a test. One problem, for example, is when one of two or more results is selected as "better resolution" when, in fact, the result with higher support is simply due to chance variation. Most researchers, in addition, will acknowledge that of 20 conclusions of monophyly each at 0.95 chance of being correct, one of them is on the average wrong, and one does not know which one. A third problem is generally unrecognized though pervasive. Although it is perfectly understandable that multiple test problems are ignored by theoreticians requiring statistically powerful discrimination of possible clades (Miller, 1981), uncertainty is contributed when a set of two or more well-supported conclusions of monophyly must be correct at the same time, such as a statement that a family is demonstrably split into two or more monophyletic families. Given that a set of two or more conclusions of monophyly must be correct at 0.95, and the probability of the set being correct is the product of the individual probabilities of being correct, then the joint probability of two conclusions of monophyly at 0.95 being correct is only 0.90 (or about one set in 10 incorrect). Thus, individual PBAs of 0.975 (or 0.97 and 0.98) are needed for a set of two to be taken as reliable, and 0.99 each for a set of five to be acceptable as correct. Partitioning the cladogram is an

Table 3. Comparison of BP, JP, and BPP at various short branch lengths using contrived four-taxon data sets with PAUP* (BP and JP with fast heuristic, 2000 replicates) and MrBayes (datatype = standard, 300,000 generations, burnin = 1000). AB:AC:BC are ratios of synapomorphic traits unique to pairs of three terminal branches A, B, and C. BPP is clearly too high at low branch lengths in this simulation.

AB:AC:BC	BCI	BP	JP	BPP
1:0:0	0.67	1	0	0.79
2:0:0	0.89	1	1	0.96
2:1:0	0.74	0.75	0.66	0.83
2:1:1	0.59	0.59	0.50	0.69
3:0:0	0.96	1	1	0.99
3:1:0	0.89	0.85	0.75	0.97
3:1:1	0.79	0.77	0.61	0.91
3:2:0	0.79	0.69	0.60	0.84
3:2:1	0.68	0.63	0.58	0.77
3:2:2	0.57	0.52	0.50	0.64
4:0:0	0.99	1	1	1
4:1:0	0.95	0.95	0.79	0.99

initial means of eliminating lineages that need not be considered as part of a set.

In statistics, likelihoods of raw data are particular manifestations of multiple events, e.g., in 100 tosses, a coin came up heads 45 times, and thus the likelihood of heads is 45% for that coin, while probabilities are ideal generalizations of expectations, e.g., a fair coin has a 50% probability of coming up heads. Frequentist methods of dealing with multiple tests, such as Bonferroni or sequential Bonferroni correction (Holm, 1979; Hochberg, 1988), control of False Discovery Rate (Benjamini & Hochberg, 1995), or new "sharpened" methods (Hochberg, 1988; Verhoeven et al., 2005) that take into account the nonuniform distributions of confidence intervals generated by phylogenetic signal, convert likelihoods to P values and examine how close they come to a separately determined (for instance by maximum likelihood) probability required for acceptance as correct. The present method follows the Bayesian convention of converting likelihoods to probabilities whenever they may be taken to well approximate the ideal, generalized expectation, such when information is deemed sufficient or, as in this case, likelihoods are demonstrably non-null, e.g., BPs and BPPs greater than 0.50 are seldom obtained by random data under parametric bootstrap (Sanderson & Wojciechowski, 2000; but see Dolphin et al., 2000).

Two branches of a tree are, of course, dependent on each other to some extent through interpretation of the data. But, after the optimum tree is settled on, any observation that the uncertainty attributed to one branch arrangement (as contrary data as opposed to lack of data) affecting the other BA may be viewed as an artifact of small taxon sampling. The data are independent (orthogonal), even when BAs are occasionally not. The joint probability reflects this, and, like Bonferroni correction (Rice, 1989), the present method does not require independence of branch arrangements because the data are independent and may be extended to data sets with more OTUs (operational taxonomic units) as a kind of pseudocount or reverse bootstrap.

As to conflict of branch arrangements, if NNI were the only results of alternatives to optimal BAs, then no lineage would change in composition, i.e., of ((AB)C)D, then C would not embed itself inside lineage A or B or D but would only switch places with A or B. If all nests in the cladogram, however, were strongly supported, e.g., by moderate to high PBAs, then C would have to crack two or more nested BAs to jump across the cladogram and modify another lineage. The chances of that are powers of 0.05 for 0.95 PBAs. Thus, a cladogram of only BAs with 0.95 and above support is sufficiently nested for probabilistic calculations by this argument and by independence of data. Using IRI with BAs of less than 0.95 is justified in the logic that (1) such BAs are probably due to signal because they are above 0.50 and thus privileged (Efron, 2005), and (2) reliability of monophyly should increase with phylogenetic distance.

The single *rbcL* tree of Malpighiales from Savolainen et al. (2000) as presented by Chase et al. (2000) showed the traditional Euphorbiaceae split into five families (indicated in Fig. 1). These and only these are the focus of this study, and these are subject to multiple test strictures. The only exception is the possible splitting of two family lineages by a third intermediate lineage (e.g., sister to one of two families) to ensure molecular support as separate families, as discussed in Step Seven.

STEP THREE

Preselect conclusions of monophyly to test, prioritized to break ties. Choose those lineages that must be viewed as a set for the conclusion of joint monophyly. In this case, we choose the five families of Step Two: (1) Euphorbiaceae (s. str.), (2) Pseudanthaceae, (3) Putranjivaceae, (4) Pandanaceae, and (5) Phyllanthaceae (see Fig. 1). Here, the most taxon-rich (in the cladogram) lineages are selected as most important and are prioritized as above. Thus, if 0.95 was the highest level of support among them, and there were two or more, then Euphorbiaceae s. str. would be the one supported at that level, and all other branch arrangements would be considered speculative. If Euphorbiaceae s. str. was not supported at 0.95, then the next in priority would be the one considered reliable. One may wonder that only one of several conclusions of monophyly would be acceptable when all have the same support, but over the course of several publications, the sets accepted would be wrong much more often than one out of 20 sets. Preselection and prioritization are thus important.

STEP FOUR

Impose a penalty for unaccounted assumptions. It is clear that there remains considerable uncertainty not reflected in BA support values given in the literature. I can only explain this as a function of three preconceptions on the part of phylogeneticists: (1) Speculation is presently considered more important than consolidation of well-grounded theory, and such investigation necessarily involves possibly critical assumptions and choice of statistical discriminative power over reliability. (2) The likelihood principle indicates that the probability of the data given the

hypothesis is the same as the probability of the hypothesis given the data, and therefore one may assume (wrongly) that the data as presented in the aligned sequence contain all the information needed to evaluate the hypothesis (given a model). (3) The probability of a given branch arrangement being correct is simply presented as the posterior probability given by Bayes' Formula. The last two are simplistic in the extreme, ignoring the necessity that any relevant factors not included in the data or model must be attended to as a joint probability (the product) of the chance all assumptions are correct. Thus, the final product of a Bayesian analysis is properly not the posterior probability but the Bayes' Solution (Kendall & Buckland, 1971), which takes into account and minimizes risk, such as uncertainty contributed by assumptions not dealt with in the method, model, or data.

In the late 1970s and early 1980s, cladistics initially presented "optimality-alone" as a justification for choosing a particular tree, in that scientists supposedly always choose the simplest answer, the one that "converges on the truth," or that which is least falsifiable. The obvious objection has now generally been accepted, namely that a branch arrangement of interest in the best tree may be contradicted in many respects by the next several best trees, which may be only slightly less optimal. With molecular data, abundant statistical support is available through bootstrapping and Bayesian posterior probabilities. Such support is sometimes quite strong. Unfortunately, the old criterion of optimality alone has been shifted from the optimal tree to other assumptions, such as the sequence alignment, for which there may be other slightly less optimal alignments, each of which may produce a different tree (Giribet et al., 2002). A recently devised method of estimating reliability of both alignment and phylogenetic trees (Redelings & Suchard, 2005) has much promise, but the literature lacks a joint probability assessment of branch arrangement and alignment reliability. In addition, using a different, less "optimal" outgroup may produce a different branch arrangement or different support measure for an arrangement of interest. In the case that several outgroups are used, the branch support for both the immediate outgroup and the tree as sister lineages could be used in a joint probability measure for a branch arrangement when the next nearest outgroup affects the result, but this kind of modified branch support value is not to be expected from the literature, and in this respect, too, the tree, however well supported prima facie, is to some extent merely "optimal."

I have examined a selection of recent phylogenetic papers and found (Zander, 2005) more than 100 biological, methodological, and computational assumptions (or contingencies) cited by authors as problematic. These assumptions are not commonly included as sources of uncertainty reflected in support values in the final cladogram as published. Examples, in addition to the egregious alignment and outgroup problems above, include: insufficient or polyphyletic exemplars, wrong gap costs, differential lineage sorting, hybridy or polyploidy, lack of clocklike behavior, rates other than gamma-distributed, differences between results of "total evidence" and evaluations based on separate gene studies, too few generations in MCMC analysis or of bootstrap replicates, possible strong selection pressure on strongly conserved non-coding sequences and persistent pseudogenes, too few exemplars, inclusion or exclusion of fossil evidence, endogenous retroviruses, gene conversion, high evolution rates, self-correction of flawed DNA, paralogy, codon bias, chloroplast capture and other horizontal gene flow, novel clades, among-lineage and among-site heterogeneities, saturation, differences when including or excluding third nucleotide position, lack of vouchers, choice of ACCTRAN or DELTRAN in PAUP*, long-branch attractions, unusual noise, differences between results from different analytic programs, sample error, and model insufficiency. Most of the 100, doubtless, are inapplicable or too improbable in any one particular instance to affect the PBA. The uncertainty may be considerable from all remaining assumptions, and some correction is necessary, even ad hoc. Consider the following: if only 10 of 100+ assumptions apply to one branch arrangement of interest and each of these assumptions were wrong and changed the branch arrangement only one out of 1000 times, then the contributed uncertainty is $10 \times 1/1000 = 1/100$, or 1% of the PBA. At this point, a penalty of 0.01, or 20% of the window of reliability, can be levied without qualm. If there are additional sources of uncertainty relevant to only the branch arrangement of interest, such as suspicion of misidentification, an additional judicious penalty can be set. A penalty of 0.01 on six chained internodes, each of 1.00 BPP, means that the chance of all branch arrangements being correct at once is reduced to 0.94. Thus, if corrected for unaccounted assumptions, no cladogram of seven or more terminal taxa can be entirely reliable. Figure 2 shows branch arrangements with BPs converted to BCIs and each reduced by 0.01 for unaccounted assumptions. This is then taken as the working PBA.

This penalty is certainly a ballpark estimate that may be changed by the user, and it has been accused of being ad hoc by reviewers. There is, however, no alternative correction for second-level users of

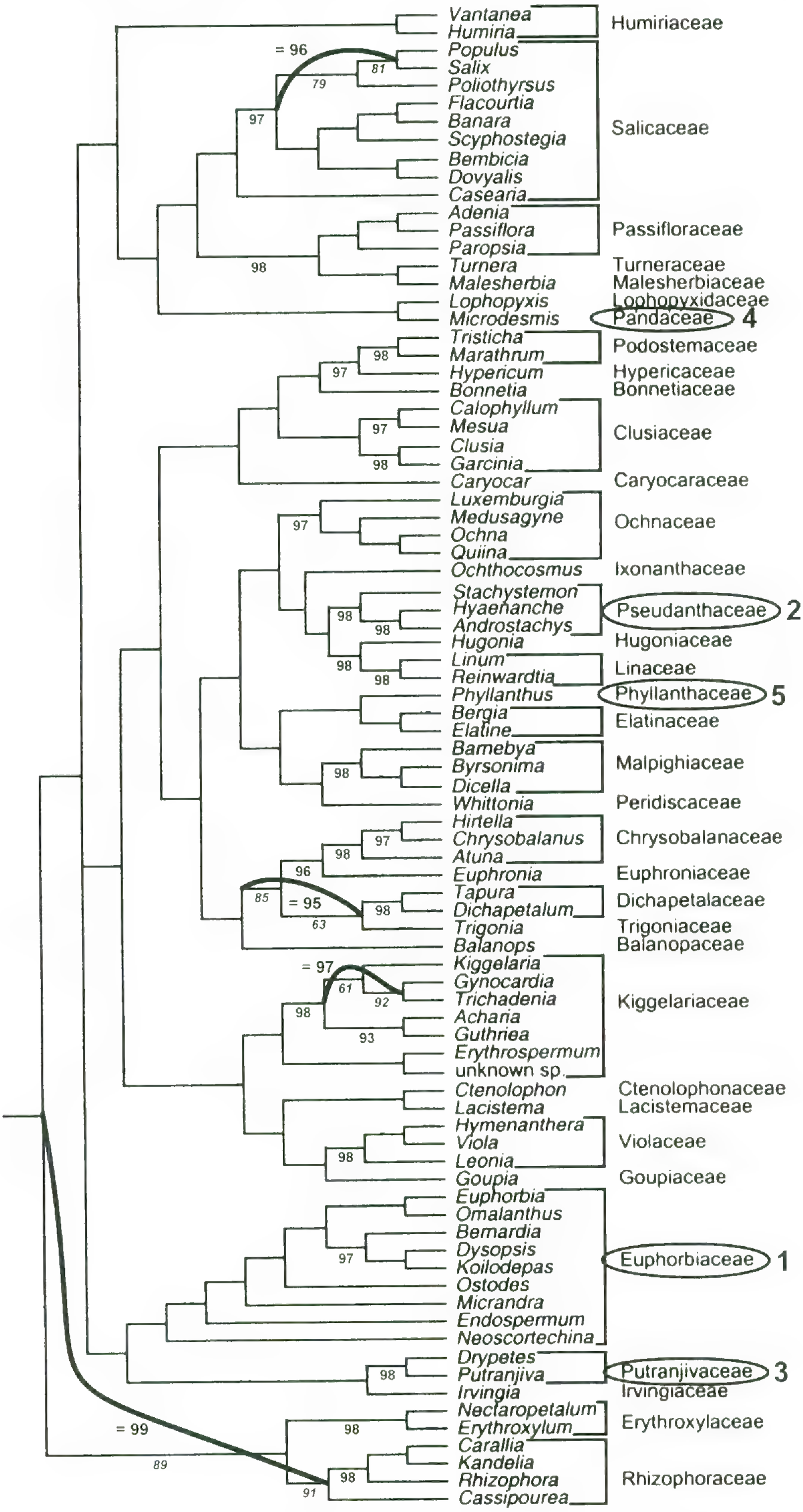

Figure 2. Malpighiales tree after conversion of bootstrap proportions into binomial credible intervals and subtraction of 0.01 penalty for unaccounted assumptions, these taken as equivalent to probability of branch arrangements being correct. All possible contiguous internodes with less than 0.95 PBA are fused into one at 0.95 or above with formula for implied reliability (curved lines). All remaining values less than 0.95 are deleted. (Modified from Chase et al., 2000; reprinted with permission.)

published analyses. Instead of the penalty for unaccounted assumptions, one can either reject all even slightly unreasonable molecular results as too speculative, or redo molecular analyses offering contrary solutions to accepted monophyly, and test whether the branch arrangements of interest are robust to reasonable variation in all relevant assumptions. One might note that accepting many assumptions, because if false they would not significantly affect any branch arrangements, was also ad hoc when statistical phylogenetic analysis began; however, the practice has been embedded in the analytic method for the last 30 years.

STEP FIVE

Combine two or more contiguous branch arrangements of PBAs of 0.50 to 0.94 into one of 0.95 or higher using the Formula for Implied Reliable Internodes (FIRI). Basically, the simplest probabilistic calculation of the chance that at least one of two or more events will happen is one minus the product of the chances they will not happen.

$$\begin{aligned} PBA_{implied} = 1 - ((1 - PBA_{internode\ 1}) \\ \times (1 - PBA_{internode\ 2}) \ldots \\ \times (1 - PBA_{internode\ n})) \end{aligned}$$

In phylogenetics, this is equivalent to the chance that one (or more) of two chained branch arrangements is correct, or (more easily calculable) one minus the product of chances that each is incorrect, where the chance of being incorrect is one minus the PBA for the branch arrangement. For example, the chance of at least one of two chained internodes at 0.81 and 0.79 PBA being correct is $1 - (0.19 \times 0.21)$, or 0.96. Compare this to the chance both are correct at the same time (as a set), which is the product, or 0.64. There is clearly a large difference when depending on the resolved branch arrangement for support of a biogeographic hypothesis. Do not use this formula with branch arrangements of < 0.50 BP or BPP or BCI, because support in those cases may be due to chance alone and the additional apparent certainty though small is inapplicable. If all branch arrangements that reach 0.95 or greater using this formula are dealt with and those that reach less than 0.95 are collapsed, a cladogram (or partition) with only branch arrangements of 0.95 or more is obtained (Figs. 3 and 4).

STEP SIX

Make corrections for multiple tests. Figure 3 shows no support for Euphorbiaceae s. str., but PBAs of 0.98 for both Pseudanthaceae and Putranjivaceae, and tentative 1.00 for Pandanaceae and Phyllanthaceae of one exemplar each. The product of the support values for the last four is 0.96, so we have acceptable monophyly of each lineage in the set of four lineages, which, however, are all basal branches when considering the molecular data set alone. There is no reliable phylogenetic structure.

Figure 4 of the operatively transformed Werner et al. (2004) cladogram shows many branch arrangements at 0.99 probability. For any particular previous hypothesis of relationship that is to be tested, up to five of these BAs can be true as a set. These should be selected from only those with 0.50 (or dubious) morphological priors, and not those with 0.01 priors because these are clearly contrary to accepted use and without alternative morphological traits supportive of the molecular BA. One can involve as a set as many as needed from those that are morphologically uncontested (therefore assigned 0.99+ PBA when using Bayes' Formula).

STEP SEVEN

With molecular data alone, reliable demonstration of paraphyly will split a taxonomic group, i.e., distinguish two groups as warranting separate names. In the case of several exemplars, such as splitting a genus or higher taxonomic category, a distance of two internodes reliable as a set with an intermediate lineage not related to either (morphologically or molecular with a distinction greater than mere monophyly) is needed. The first distance distinguishes the terminal isolate of the two as distinct from the disparate sister lineage; the second establishes the second isolate that is lowermost in the tree as not being merely a basal branch (and therefore potentially monophyletic). Conditions for distinguishing taxonomic groups based on molecular data alone, without reference to morphology, are not established. There is progress being made in species delimitation using multiple molecular samples (Pons et al., 2006), but molecular data as used in the literature are generally sparse, represented by one or a very few exemplars, and are without the extensive analysis of variation represented by morphology-based taxonomy. Two lineages, separated by a third reliable lineage, must both be true at the same time (as a set), and so the product of their support values should reach 0.95. Two lineages among the split portions of the Euphorbiaceae in Figure 3 reach 0.98 and are reliable as a set. None of the five lineages, however, are reliably other than a basal branch of the Euphorbiaceae. Their status as separate families remains entirely morphological (based on this one publication). The high (0.97) PBA support for mono-

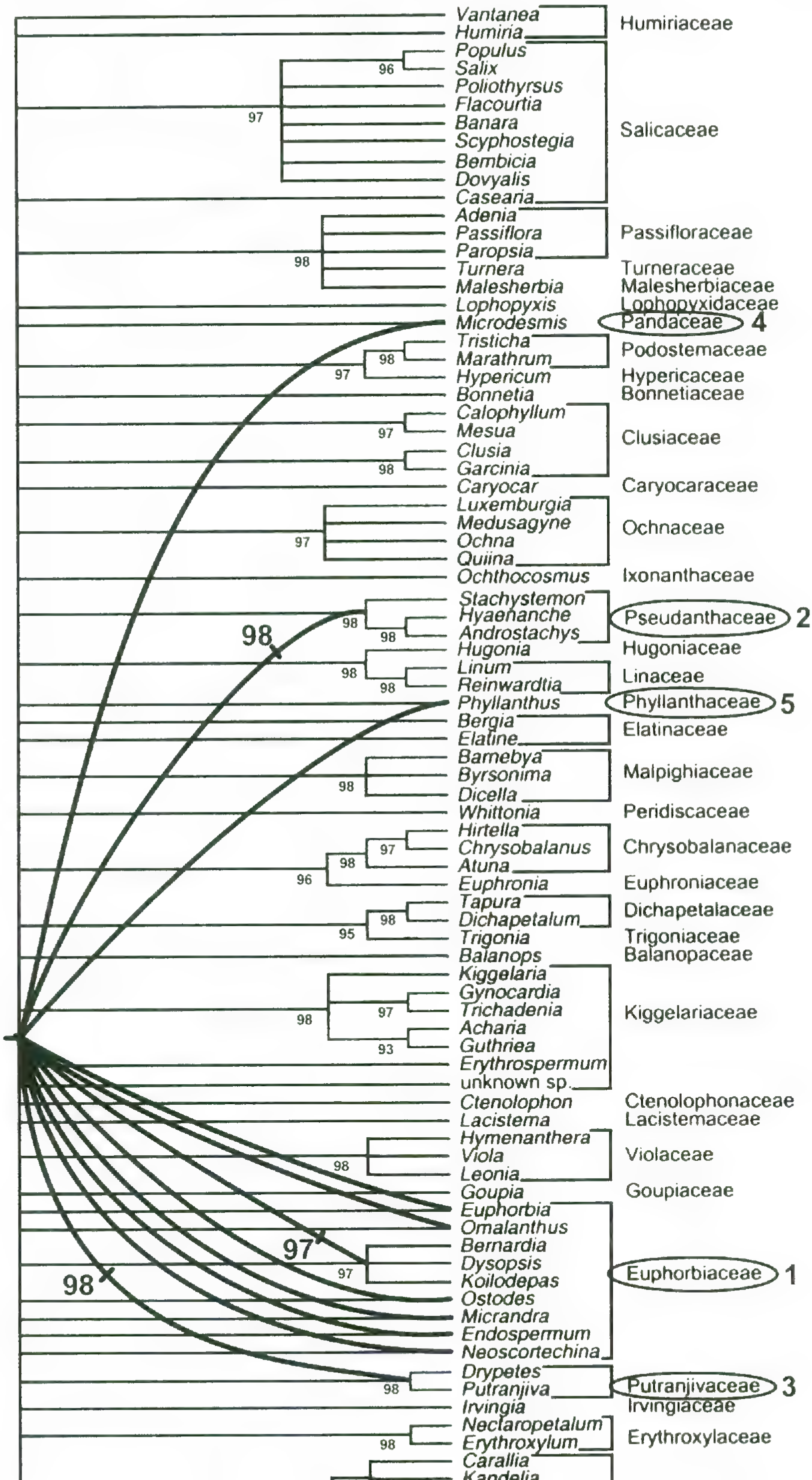

Figure 3. Operative transform of Malpighiales tree with all branch arrangements less than 0.95 PBA collapsed. The five families of Euphorbiaceae sensu Webster are found to be arranged without reliable phylogenetic structure (as multifurcative sister groups). No family is isolated from the traditional Euphorbiaceae by a disparate intermediate lineage, and thus only non-molecular traits sustain them as distinct. Two families are each monophyletic as a set (joint probability = 0.96), while the three exemplars in Euphorbiaceae s. str. united at 0.97 must be viewed as speculative (joint probability with other two conclusions of monophyly is 0.93). (Modified from Chase et al., 2000; reprinted with permission.)

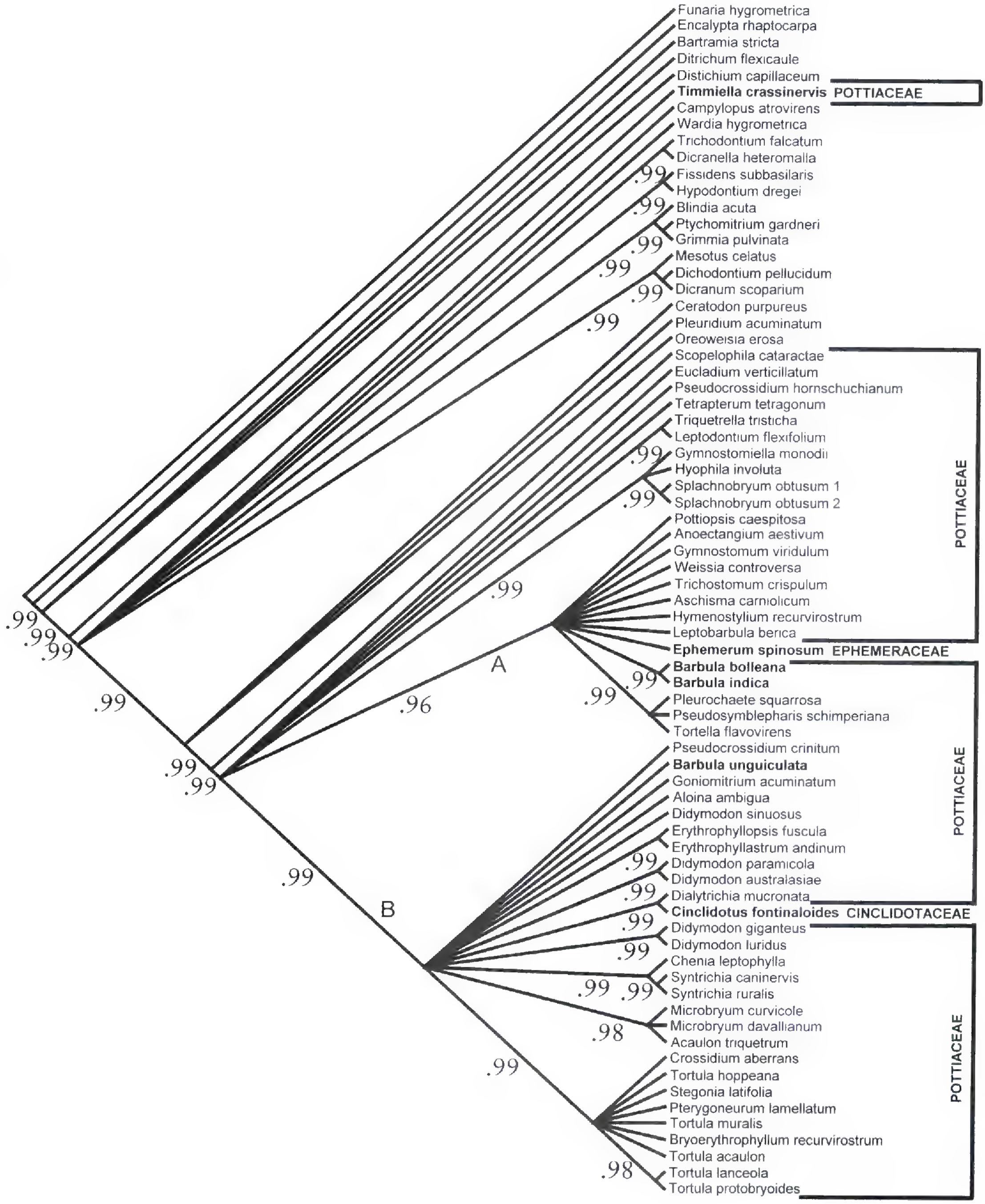

Figure 4. Operative transform of the *rps4* tree of Pottiaceae and associated families of Werner et al. (2004). *Timmiella* is retained in the Pottiaceae by recourse to Dollo's Rule. *Ephemerum* (Ephemeraceae) and *Cinclidotus* (Cinclidotaceae) are excluded from the Pottiaceae, which is retained in spite of being paraphyletic, justification being similar phenotype and survival strategy throughout the family leading to a single type of functional evolution.

phyly of a portion of Euphorbiaceae s. str. must be regarded as speculation, as 0.98, 0.98, and 0.97 are not reliable as a set (product = 0.93).

The same publication (Savolainen et al., 2000) also split the Flacourtiaceae into Salicaceae and Kiggelariaceae, which is an entirely different multiple test problem. Examination of Figure 3 indicates that the Salicaceae and Kiggelariaceae, though widely separated in the original cladogram (Fig. 1), are doubtfully more than sister groups. Molecular coherence of large

internal portions of these families plus phytochemical distinctions combine, however, in rather reliably distinguishing them.

The stricture of Step Seven is true only when recognizing paraphyletic taxa proves no purpose. There may well be situations in which two or more patristically distant lineages are not functionally evolutionarily divergent (as inferred from phenome) though divergent in time (as tracked by neutral DNA base changes). In that case, such two or more lineages may be considered taxonomically one, with a functionally divergent separate taxon emerging from anywhere within that single ancestral group (phylogenetically well-structured species, genus, or family). An example would be an ancestral population isolated into two populations, then both continue to accumulate neutral mutations but retain the same phenome, a second population is isolated from the terminal population and diverges in phenome–environmental interaction through selection while the ancestral population is unchanged except for continued accumulation of neutral mutations. All three groups are different if viewed from a neutralist standpoint, yet the ancestral population and the first isolate are phenomically identical though paraphyletic. There may even be several phenomically evolutionarily divergent populations separating a surviving ancestral population and a daughter group with identical evolutionary strategies, which would argue against distinction of the two at higher taxonomic levels. A two-internode distance based on genes responding to recombination is only equivalent to a minimum distance of two genetically isolated panmictic or tokogenetic populations, while with matrilineally inherited (chloroplast or mitochondrial) DNA it is equivalent to a pedigree of a minimum of two ancestral individuals.

STEP EIGHT

Use Bayes' Formula to combine separately published branch arrangements based on different data. When two or more published trees are relevant to a BA of interest, select a branch arrangement of three taxa or lineages of interest, commonly from the most well-supported and complex cladogram. This one is then the "nominal branch arrangement." We ask, now, is this particular arrangement, say (AB)C, on the whole supported or falsified by previous studies? Bayes' Formula may be used. Likelihoods of model conditions have been previously addressed in computerized analysis of the published study, so the Formula as applied here is now a simple proportion. All probabilities must be conditional on being expressed as support for (AB)C, and this must be estimated from the different published studies, even when lineages A, B, and C are presented as a bush, i.e., as (ABC). Any introduction of uncertainty must be addressed, and support for (AC)B of 0.70 can be represented as support of 0.30 for (AB)C, though this is, of course, generous. If (AB)C is supported by a value given as merely less than 0.50, 0.42 (midway between 0.33 and 0.50) is an appropriate assignment, though less than generous.

In cladograms with some branch arrangements collapsed because the support is less than 0.50, the multifurcation (ABC) should be interpreted as 0.33 support for (AB)C because, if the same data set does well support many branch arrangements in other areas of the cladogram, then (ABC) is evidence of a hard polytomy or resolved branch arrangement indistinguishable from such and cannot be ignored. In cases when support values may have been generated by randomness in the data set, such low support values can be ignored only if they tend to increase certainty (see Step Six); any decrease of certainty must be addressed in Step Eight.

The Bayes' Formula expresses a percentage when the joint probability (chance of the set of prior and nominal probability being both correct) is divided by a normalizing constant (chance of the set being correct plus the chance that the set is incorrect, and therefore summing all possible cases). Because of the normalizing constant, the chance of a hypothesis being correct increases with additional probabilities greater than 0.50. The Bayes' Formula is commonly used when the prior is a subjective measure of prior knowledge, but here the prior is entirely empirical, being the result of a previous analysis. Given that both prior and nominal branch arrangements must evaluate probabilistically (AB)C because the Formula is conditional on (AB)C being correct at some level of certainty, the posterior probability for (AB)C is:

$$\frac{(\text{Prior correct} \times \text{Nominal correct})}{((\text{Prior correct} \times \text{Nominal correct}) + (\text{Prior incorrect} \times \text{Nominal incorrect}))}$$

or

$$\frac{(PBA_{\text{paper 1}} \times PBA_{\text{paper 2}})}{((PBA_{\text{paper 1}} \times PBA_{\text{paper 2}}) + ((1 \quad PBA_{\text{paper 1}}) \times (1 \quad PBA_{\text{paper 2}})))}$$

This may be continued with several papers, with the posterior probability of one calculation serving as the prior (or "paper 1") of the next.

Inclusion of evidence from morphology (or chemistry, ultrastructure, etc.) cannot be ignored, although, with addition of data from several molecular data sets,

it justifiably may be overwhelmed by successive Bayesian analyses if contrary. When exact likelihoods are unavailable, Bayesian subjective priors may be assigned. If the morphological branch arrangement is "uncontested" or "accepted," and it agrees with the nominal (molecular) branch arrangement, assign it a prior of 0.99. If it disagrees, assign it 0.01. If the morphological branch arrangement is equivocal, assign a 0.50 prior; with Bayes' Formula, a 0.50 value does not change the posterior probability of the nominal branch arrangement.

Imaginary examples for piecemeal "total evidence" from separately published studies are here presented using Bayes' Formula with each posterior probability treated as the prior for the next calculation:

(1) The nominal branch arrangement of a molecularly derived conclusion of monophyly (AB)C supported by probability 0.80 is combined with a second result for (AB)C of 0.70: $(0.80 \times 0.70)/((0.80 \times 0.70) + (0.20 \times 0.30)) = \mathbf{0.90}$ as BPP.

(2) A third, morphological data set is equivocal between two branch arrangements, (AB)C and (AC)B. Using 0.90 as a prior, we combine it with 0.50, which is the probability from the equivocal data that either (AB)C or (AC)B is correct: $(0.90 \times 0.50)/((0.90 \times 0.50) + (0.10 \times 0.50)) = \mathbf{0.90}$, and the posterior probability stays the same.

(3) A fourth molecular data set supports the contrary tree (BC)A at 0.80, and therefore (AB)C is at most supported by 0.20: $(0.90 \times 0.20)/((0.90 \times 0.20) + (0.10 \times 0.80)) = \mathbf{0.70}$ as BPP that (AB)C is correct.

(4) A fifth data set with many other branch arrangements well resolved presents a bush (ABC) for the three taxa of interest, and (AB)C can be assigned a fail-safe 0.33: $(0.70 \times 0.33)/(0.70 \times 0.33) + (0.30 \times 0.67)) = \mathbf{0.54}$ BPP.

(5) A sixth data set with (AB)C at 0.80: $(0.54 \times 0.80)/((0.54 \times 0.80) + (0.46 \times 0.20)) = \mathbf{0.82}$ BPP.

(6) A seventh data set with (AB)C at 0.80 increases the BPP to **0.95**, which indicates a reliable conclusion of monophyly based on all the data. Bayesians may or may not select beforehand a stopping rule for sequential procedures, but in any case many tests are needed to decide if the data are adequate for reliable resolution. One should not, however, expect the great variation expressed in this imaginary set of examples. Modern molecular analyses with large data sets from multiple loci, using robust technique, often produce very well-supported molecular trees.

It appears in Figure 4 that species of *Barbula* Hedw. are strongly supported as split into two groups: *B. bolleana* (Müll. Hal.) Broth. and *B. indica* (Hook.) Spreng. in Steudel associated with mostly species of subfamily Trichostomoideae (lineage "A" in Fig. 4), and *B. unguiculata* Hedw. with mostly species of subfamily Barbuloideae (basal branches of "B"). The Trichostomoideae is held together at 0.96 probability, and the other BA at 0.99; thus a joint probability of 0.95 supports a hypothesis that the two groups of *Barbula* species are indeed separated by two internodes. Because the Werner et al. (2004) study used chloroplast DNA, which is matrilineally inherited as a haplotype, the exemplars represent individuals, not species. Genetic isolation begins with the individual with chloroplast and mitochondrial DNA. There are thus potentially as few as two ancestral individuals in the pedigree separating the two groups. On the other hand, the *Barbula* species in the Trichostomoideae have the plane leaves characteristic of the Trichostomoideae, which provides a 0.95 morphological prior yielding a 0.99 probability by Bayes' Formula that they do belong in the Trichostomoideae, while the other species of *Barbula* has the recurved margins of the Barbuloideae. This evaluation, thus, supports reclassification of plane-leaved species of *Barbula* with Trichostomoideae, although corroboration with additional study would be helpful.

STEP NINE

Examine the potential value of recognizing paraphyletic taxa by tempering strict monophyly with evidence for taxic unity based on inferred functional evolution and non-dichotomous evolutionary processes. Avoiding paraphyletic classificatory elements in favor of strict monophyly has been recently identified as a major problem with modern systematics (e.g., Brummitt, 2003, 2006; Nordal & Stedje, 2005; Hörlandl, 2006). Of three taxa or exemplar specimens, modern molecular phylogenetic analysis asks: "Which two share a recent event of genetic isolation, and how probable is this event?" After analysis, morphological traits are now commonly mapped on a molecular tree of such inferred events and conclusions made about morphological trait evolution, often involving discovery of abundant morphological homoplasy and convergence. The problem involves neutralist evolutionary philosophies coupled with cladistic insistence on strict monophyly.

Any long period of isolation of two or more populations results in accumulation of theoretically neutral DNA base changes in each population that clearly matches traditional cladistic speciation (two new taxa from one ancestral taxon, which then disappears). Requirement of strict monophyly assuming the biological species concept (involving genetic isolation) implies that any phylogenetic structure may be documented as a dichotomous tree down to basic tokogenetic or panmictic units. Many species, espe-

cially plants, have molecular phylogenetic structure associated with full or partial genetic isolation of populations, but such multiplex species can retain an essential morphological and ecological unity, for instance through processes like stabilizing selection. If paraphyletic groups may be described as having a unity in descent with modification by showing a distinctive morphology with reproductive and survival strategies, then they should be recognized in classification as single taxa. That such hypotheses of adaptation and selection sometimes have alternative explanations (Gould & Lewontin, 1979), albeit less persuading, is part of the incremental nature of scientific analysis.

Classification based solely on monophyletic groups (Ebach et al., 2006) is then crippled in explanatory power when a portion of such a group is nothing more than an isolated element of a greater assemblage that is biologically better recognized as a unit, and has practical value (Raven, 1974) across science. By extension, taxonomic splitting of genera and families to fit a relentless pursuit of molecular pedigree cannot produce concepts of taxa that document facts or hypothetical explanations of evolutionary process that involve multiple populations, but instead produce just a chart of inferred molecular ancestral relationships. Strict monophyly splits evolutionary units of multiple populations into cryptic species of no particular value except possession of a line of neutral mutations. Such finely dissected lineages are of dubious value in biodiversity triage and mask truly important differences among species needing conservation protection.

Computer-generated schemes of monophyly, either as reflected in morphological cladograms sorting all traits, or morphological traits or taxa mapped to molecular cladograms based on nuclear or organellar genes, cannot address evolutionary change adequately. When classifying evolutionarily divergent taxa, it is best to continue to recognize paraphyletic groups when their functional differences are not involved. For example, the Bayesian *rps4* cladogram of genera of the Pottiaceae (Bryopsida) and related genera of Werner et al. (2004) showed the family Ephemeraceae embedded deeply (two internodes, 0.95 joint probability) in the Pottiaceae (Fig. 4). Functional evolution is rather different for the two somewhat morphologically different families, with Ephemeraceae adapted to avoidance of harsh conditions while the Pottiaceae generally tolerates them. Though apparently molecularly embedded in the Pottiaceae, the Ephemeraceae should be recognized as a family with a separate evolutionary unity, leaving the Pottiaceae paraphyletic. Likewise the Cinclidotaceae, adapted to aquatic habitats, rates family status distinct from the paraphyletic Pottiaceae though well embedded (three internodes, joint probability 0.97). The presence of *Goniomitrium* J. D. Hook. & Wils. (Funariaceae) was apparently based on a misidentification of an *Acaulon* Müll. Hal. species (Werner et al., 2007) and is here discounted. That systematists should accept at least occasional non-dichotomous evolution in classification has been well discussed by Hörandl (2006) in the context of recognizing paraphyly when appropriate.

In addition, one should check for contravention of Dollo's Rule against re-evolution of complex traits requiring consideration of reticulation and possible evolutionary Lazarus taxa. There are many examples of complex deep homologies re-expressed higher in a phylogenetic tree. These include: apparent re-evolution of wings in wingless walking sticks (mantids), shell coiling in snails, single eyes (a pleisiomorphic trait) in brine shrimp, eyes in eyeless copepods, modes of vulva formation in nematodes, ancestral features of the lateral lines, muscles, and gill rakers of cichlid fishes, teeth in chickens (laboratory), occurrence of a second molar in lynx (reviewed by Zander, 2006), and, recently, patterns of water storage tissue in *Adenia* Forrsk. (Passifloraceae; Hearn, 2006). These phenomena have been explained by gene cluster silencing (through, for example, cytidine methylation or histone deacetylation), surviving millions of years with little degradation (Fryer, 1999; Lynch & Force, 2000; Collin & Cipriani, 2003), and subsequent reactivation. Convergent evolution of complex organs and other massive homoplasy at a particular point become extremely improbable and may be taken as evidence of evolutionary Lazarus taxa (as opposed to geological Lazarus taxa that reappear after long periods in more recent strata). If identified correctly, reticulate evolution is the proper explanation, and taxonomic splitting is unwarranted.

As example, the same Bayesian *rps4* cladogram of genera of the Pottiaceae and related genera (Werner et al., 2004) showed *Timmiella* to occur toward the base of the tree far from the Pottiaceae proper. *Timmiella* is morphologically pleisiomorphic to the Pottiaceae, and it is found at the base of the morphological tree generated by Zander's (1993) cladistic analysis. This genus is rich in the characters of the family, possesses the distinctive twisted peristome of the family, and is not morphologically similar to any family nearby in the Werner et al. (2004) cladogram. A transformed version (Fig. 4) of the Werner et al. (2004) cladogram with all branch arrangements at 0.95 PBA or higher definitely shows *Timmiella* distinct from the Pottiaceae by three branch arrangements that are reliable as a set, with intervening lineages of other families. Although one might consider making *Timmiella* representative of a new family, when morphology is considered the cladogram contravenes Dollo's Rule,

and reticulation through gene cluster silencing and reactivation is a better and more parsimonious explanation.

An alternative explanation for the above (B. Goffinet, pers. comm.) is that lineages (other families) between *Timmiella* and Pottiaceae s. str. simply involved deactivation of the Pottiaceae traits, and there was no reactivation. In this case, however, populations comprising the sister groups of the intermediate lineages (the main line to Pottiaceae) would clearly be Pottiaceae (though gradually undergoing anagenetic changes in non-coding or evolutionarily neutral sequences), and the acceptance of intermediate lineages from a paraphyletic basal group is justified. Continued identity as one family through concatenated sister groups of other families, though more dubious than the former explanation, is equivalent to reticulation in the cladogram and can be interpreted as Lazarus-like survival. *Timmiella* is in any case justifiably retained in the Pottiaceae. The phylogenetic distance between *Timmiella* and the Pottiaceae may be considered merely three inferred events of genetic isolation following the Biological Species Concept, and it is less than the five events inferred between the base of Pottiaceae s. str. and the terminal group involving *Syntrichia* Brid. Given that tracking events of neutral evolution here involved matrilineally inherited chloroplast traits, as few as three ancestral individuals may separate *Timmiella* from the Pottiaceae s. str.; this admittedly extreme suggestion involves assumption of great clock heterogeneity.

Discussion

Optimality alone in phylogenetic analysis as justification for public presentation is rarely seen today. Philosophical justifications for optimal or shortest trees, such as "simplicity," "converging on the truth," "most parsimonious," or "least falsified," as sufficient criteria of satisfactory results in phylogenetic analysis have been replaced with various methods of gauging statistical support (with reference to the Central Limit Theorem, which basically comes from physics) for trees or branches. In addition, as noted above, resolution alone is insufficient to demonstrate reliability because random data usually produce resolved trees; length of branches alone is insufficient because large random data sets generate long branches; and agreement alone between two cladograms is insufficient because if one or both arrangements are at less than 0.50 probability (given sufficient data), then, by Bayes' Formula, the BPP must be reduced, not increased. Phylogeneticists' use of Occam's Razor (Posada & Buckley, 2004) continues to be abused, however, in that the past difficulty of evaluating the relative importance of suboptimal solutions has been repositioned to acceptance of optimal sequence alignments (analysis of Redelings & Suchard, 2005) and model selection (e.g., critique of Alfaro & Huelsenbeck, 2006), and simply assuming that all the other many assumptions associated with methods of phylogenetic reconstruction are correct or correct enough. Any use of "best" solutions when "next best" is large enough to crowd the reliability window of 0.05 is inappropriate. Although phylogenetic analysis of bryophytes using molecular data largely agrees with traditional concepts, major revisions in taxonomy have been urged for vascular plants on its basis, and clearly a revisitation of relevant molecular studies (e.g., APG II, 2003) is in order.

Huelsenbeck and Ronquist (2001) and Huelsenbeck and Immenov (2002) have described making the posterior probability of one analysis the prior for the next (in empirical Bayesian analysis) by substituting the posterior probability of the previous study prior for a non-informative prior during the next analysis. The present simple method using Bayes' Formula advocated here is, however, nonparametric, and can be used without complex analysis.

Information from both molecular and morphological studies must be dealt with, but just how is the puzzle. Morphological characters are good clues for inference of organismal evolutionary strategies for fitness and adaptation. Recognizing species and higher categories that document or hypothesize functional evolution (e.g., Robinson, 1990) or otherwise hypothetically explain the phenome and environment interaction are important in an age of rapid environmental change associated with human activity. Zander (1993) proposed a reclassification of the moss family Pottiaceae emphasizing traits of the gametophyte over those of the sporophyte, with the justification that parallel and largely identical transformation series of reduction in the sporophyte in many lineages well explains the similarities of species apportioned among traditional genera. Some but not all large genera in the family demonstrated a vast range of sporophyte reduction from peristomate, cylindrical capsules dehiscent by a lid and situated on elongate seta to nearly sessile, spherical capsules rupturing irregularly. Selective pressures on the phenotype resulting in atelochory (precinctiveness) in often patchy and harsh environments is an appropriate hypothesis accounting for this reduction. Molecular results to date commonly support to some extent Zander's new classification, but one might question whether probabilistic inferences of evolutionarily neutral ancestral traits really test these new hypotheses when neutral mutations provide only indirect and fragmented evidence of directional and

disruptive selection acting as a whole on several genetically isolated populations of a species, insistence on strict monophyly further scrambles interpretations of evolutionary process, and consideration of adaptation to particular environmental niches is generally given short shrift in modern systematics beyond mapping morphological traits on a molecular tree.

Step Nine may be the most controversial of the here recommended elements of reanalyzing published cladograms. In systematics, the major issues of our times are the phylogenetic or biological species concept versus the ecological species concept (e.g., Anderson, 1990; Grant, 1992; Pianka, 2000), the neutralist versus the selectionist positions on evolution (e.g., Ohta & Gillespie, 1996), and strict monophyly versus paraphyly (e.g., Brummitt, 2003; Ebach et al., 2006; Hörlandl, 2006). Within the scientific culture of phylogenetics, there are three commonly presented alternatives to traditional evolutionary hypotheses of adaptation and fitness involving phenome and environment. (1) Morphological phylogenetics produces a parsimonious tree or trees involving all presumably independent morphological traits, yet not all traits are evolutionarily functional. (2) With nuclear genes, molecular tracking goes right down to the nut of a tokogenetic or panmictic population. Yet above the panmictic population, there may be at times some or abundant gene flow between populations, of importance in evolution. Molecular phylogenetics is based on the idea that neutral base changes track events of genetic isolation, in practice equivalent to pan-neutralism. The phylogenetic species concept actually assumes the biological species concept, namely, it asks of three exemplar OTUs, which two probabilistically share a recent event of genetic isolation, then extends the inference to whatever the exemplar is supposed to represent. Molecular analysis of this sort should not work well with groups that are not particularly well described by the biological species concept (e.g., plants). There can be no "surviving ancestors" because as soon as a population is genetically isolated, neutral traits begin to accumulate divergently in both the surviving ancestral population and all divergent lineages. All new lines, given time, can be identified by accumulations of neutral traits whether or not their essential biorole or evolutionary strategy changes. (3) With matrilineally inherited molecular traits, such as mitochondrial and chloroplast loci, neutral base changes track the individual (e.g., "African Eve"); this is true also for patrilineal traits on Y-chromosomes, though these are seldom used in systematics. But above the individual, populations may be genetically isolated but retain phenomic and reproductive similarity and an essentially identical interaction with the environment. Some genetically isolated populations are essentially identical to each other due to, for instance, stabilizing selection on exons. Evolution in the ecological context is not directly tracked in monophyletic classifications in modern work, though some studies may also treat it as "mapped." Thus, the fan-shaped pedigrees of matrilineally inherited neutral traits will support any exemplar associated with taxonomic splitting, because the molecular analysis is biased toward excessive taxonomic atomization. The consilience of molecular and morphological systematics may be more a function of similar bias in splitting than true support for the taxa supposedly represented by the exemplars.

Exemplars often represent quite different concepts: with matrilineal inheritance of molecular traits, it is the individual; with nuclear genes, the panmictic population; and with cladograms of morphological traits, a series of all traits whether involved in functional evolution or not. In effect, an exemplar enters phylogenetic analysis as a traditional concept including inferred evolutionary process but emerges as simply a molecular genealogy.

Any family newly discovered to be deeply embedded in a lineage of another family can and should be continued to be recognized at the family level if it is considered to represent a new evolutionary direction with associated changes in morphology, reproductive strategies, and ecological niche. Strict monophyly is a classificatory shortcut having the attraction of simplicity. Inferring a process of functional evolution eventually involves a large amount of work in population and reproductive biology, biophysics (for exactly what reason does this plant have this structure?), and biosystematics. We made a good start with the Modern Synthesis, but 30 years ago got sidetracked into a simplistic methodology focused on reconstruction of genealogy. Computerized parsimony and Bayesian analyses of morphology and molecular data are, of course, helpful in classification, but an evolutionarily-based classification requires evaluation, however difficult, of descent *with* modification, not just genealogies, however detailed.

In summary, the Nine Easy Steps provide a first pass at assessing uncertainty in published phylogenetic analyses with a view to providing well-supported theory on which to base new research. Those with interest in the fundamentals involved in the question of descent alone with traits mapped on dichotomous trees (phylogenetic analysis) versus descent with modification with trees or retes reflecting hypotheses of trait change evaluated in the context of reproductive and environmental strategies and the concept of niche (evolutionary analysis) need to closely examine

the conflict between purveyors of the biological species concept (enforced by standard phylogenetic analysis) and the ecological species concept, between neutralist (including mutationist) and selectionist evolutionary philosophies, and between those requiring strict monophyly and those allowing paraphyly.

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FLORAL BIOLOGY OF *BABIANA* (IRIDACEAE: CROCOIDEAE): ADAPTIVE FLORAL RADIATION AND POLLINATION[1]

Peter Goldblatt[2] *and John C. Manning*[3]

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ABSTRACT

Field observations, floral dissections, floral nectar, and pollen load analyses of captured insects of 53 species of *Babiana* Ker Gawl. show that flowers of this geophytic genus of some 90 species of Iridaceae subfamily Crocoideae, predominantly of the southern African winter-rainfall zone, are cross pollinated by a wide range of animals. These include passerine birds and insects of four different orders, Hymenoptera (mainly Apidae), Diptera (mainly Nemestrinidae), Coleoptera (Scarabaeidae), and Lepidoptera (mainly Noctuidae). Apid pollination involves two discrete systems—passive pollination by anthophorines and native *Apis mellifera* foraging for nectar and active pollen gathering by *A. mellifera* and other Apoidea foraging for pollen. From what is known about relationships within *Babiana* and Crocoideae, it seems likely that passive pollination by anthophorines and honeybees, with nectar secreted in zygomorphic, bilabiate flowers as a reward, is the ancestral condition; it is also the most common, demonstrated for 18 and inferred for 35 more species from all three taxonomic sections of the genus. Active pollination by honeybees and female Apoidea foraging for pollen was demonstrated in one species and is inferred for four more, all of which have radially symmetric flowers and prominent anthers. Pollination by long-proboscid nemestrinids, mostly species of *Prosoeca*, is recorded for 13 species and inferred for five more, while moth pollination is recorded for one species and inferred for another two. Pollination exclusively by hopliine scarab beetles, known for six species, is associated with development of radial symmetry of the flower. Passerine bird pollination, associated with the classic syndrome of a wide floral tube, red floral pigmentation, and rigid, well-exserted stamens, occurs in two species, and is inferred for one more. Species with a bimodal system in which bees and beetles both visit and accomplish pollen transfer is known for three species. Comparing pollination systems with what is known about species relationships in *Babiana*, we infer that long-proboscid fly pollination evolved at least four times and moth pollination three times. Active pollination by pollen-collecting bees and hopliine beetle pollination also probably evolved three times each and bird pollination twice. Pollination systems are labile, and we postulate that there has been a minimum of 14 shifts in pollination system, approximately one shift for every six species. Lastly, *Babiana* species show the same correlation of morphology and floral presentation with particular sets of pollinators, described for several other genera of Iridaceae, e.g., *Gladiolus* L., *Hesperantha* Ker Gawl., and *Lapeirousia* Pourr., as well as Geraniaceae and Orchidaceae. This increases our confidence in predicting pollinators on the basis of floral presentation in other species and genera in which pollinators have not been established.

Key words: *Babiana*, Crocoideae, Iridaceae, pollination, southern Africa, winter-rainfall zone.

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Studies of the pollination ecology of the African Iridaceae have repeatedly shown that medium-sized to large genera exhibit an unusual degree of plasticity in floral morphology and that, consequently, species of each genus are pollinated by different sets of insect or avian pollinators. Moreover, the speciation and radiation of these genera are closely associated with shifts in their pollinators (Bernhardt & Goldblatt, 2000; Goldblatt et al., 2001). For example, different species of *Lapeirousia* Pourr. are pollinated by long-proboscid flies, nectar-feeding bees, or moths, or, in a few instances, a mix of bees, bee-flies, and butterflies (Goldblatt et al., 1995). The majority of species of *Gladiolus* L. appear to be pollinated primarily by nectar-feeding anthophorine bees (Goldblatt et al., 1998a), but some red-flowered species are pollinated by the large satyrid butterfly, *Aeropetes* (Johnson & Bond, 1994; Goldblatt & Manning, 2002); others are dependent on pollen-collecting female andrenid and halictid bees (or a combination of these bees and hopliine beetles) (Goldblatt et al., 1998b), long-proboscid flies, moths, or birds (Goldblatt & Manning, 1999, 2002; Goldblatt et al., 1999). Consequently, adaptive radiation of pollination-related floral characters appears to have played a prominent role in evolution and speciation within African Iridaceae.

The southern African genus *Babiana* Ker Gawl., with some 90 species of corm-bearing geophytes (Goldblatt & Manning, 2007 and unpublished data), is one of the few remaining larger genera of southern African Iridaceae for which data on pollination ecology are deficient. Floral variation in the genus is, however, extensive, and we undertook this study of the pollination ecology of *Babiana* to determine to what extent pollination mechanisms correlate with floral morphology and phylogeny. *Babiana* is exceptional in subfamily Crocoideae in the dominance of blue flowers among the species. In addition, it has radiated most extensively in the arid, winter-rainfall semi-desert areas of western southern Africa that surround the Cape Floristic Region.

Methods

Inflorescence phenology and floral life span

Direct observations are presented on 54 taxa of *Babiana* (53 species and one subspecies) made in the field from 1993 to 2004 (Table 1) across the entire geographic range of the genus. Supplementary observations were made on living collections at Kirstenbosch Botanic Gardens, Cape Town, South Africa, and in Portland, Oregon, U.S.A. The taxonomy followed is that of Goldblatt and Manning (2007). Field observations were made in the southern winter and spring at sites in southern Africa, in the Northern and Western Cape Provinces of South Africa, which have a Mediterranean-type climate with wet winters and dry summers. Observations of insect foraging involved 4–10 hours per plant species and included recording of floral attractants (pigmentation, scent), rewards (nectar, pollen), the mode and timing of anthesis (opening of individual buds), daily phenology, anther dehiscence, expansion of stigmatic lobes, the behavior of insects on the flower, and the taxonomic diversity of floral foragers. Floral scent was noted in the field and in cultivated plants. Scents too weak to be detected by the human nose were recorded after individual flowers were picked and placed in clean, lidded glass jars and stored in a warm place. The contents of each jar were smelled after a minimum of 60 minutes (Buchmann, 1983).

Flower longevity estimates were made using greenhouse-cultivated plants. Flowers were identified with dated jeweler's tags on the day they opened and were monitored daily until the perianth collapsed, at which point the tag was again dated and then removed.

Nectar analysis

Standing crop nectar volume measurements were taken primarily from unbagged flowers in the field and reflect both rates of secretion and depletion. Nectar volume is expected to be lower in unbagged versus bagged flowers, but sampling of nectar of unbagged flowers in populations being visited by pollinators reflects a realistic situation that confronts a pollinator in the field. To collect nectar, whole flowers were picked, and nectar was withdrawn from the base of the perianth tube with 3-µl capillary tubes after separating the ovary from the perianth base. The percentage of sucrose equivalents in fresh nectar was measured in the field using a Bellingham and Stanley (Tunbridge Wells, United Kingdom) handheld refractometer (0%–50%) from five or more individuals per population, unless fewer individuals were available. Additional nectar samples were dried on Whatman filter paper no. 1 (Whatman International Ltd., Springfield Mill, United Kingdom) and sent to B.-E. van Wyk, Rand Afrikaans University, Johannesburg, for high-performance liquid chromatography nectar chemistry analysis (van Wyk et al., 1993).

Compatibility relations

Compatibility relationships of *Babiana* species were examined in some greenhouse-cultivated plants using one to three genets each of eight species. Plants were isolated from insect foragers with nylon netting. Individual flower buds were tagged with jeweler's tags. For

one set of flowers, we self-pollinated the stigma by hand with the contents of dehiscent anthers from the same flower (autogamy) or flowers on the same inflorescence (geitonogamy). The pollinated flowers were then checked after 42 days (six weeks) to observe capsule production. For a second set of flowers, we kept plants isolated from insect foragers but did not hand-manipulate blooms in any way in order to determine whether mechanical self-pollination (autogamy) occurs in flowers in the absence of pollen vectors.

INSECT OBSERVATION AND POLLEN LOAD ANALYSES

Observations of insects on *Babiana* flowers included whether insects contacted anthers and stigmas while foraging. Only insects observed probing the floral tube or brushing the anthers or stigmas were captured and killed in a jar using ethyl acetate vapor. To prevent contamination of the body of an insect with pollen carried by another in the same jar, each insect was wrapped in tissue as soon as it was immobilized. Body and proboscis lengths were recorded from captured specimens. Capturing some insects (especially long-proboscid flies) at some sites appeared to reduce the population significantly, and we therefore killed as few insects as necessary to obtain specimens for identification and pollen load analysis. Removal of pollen from insect bodies after they were pinned involved gently scraping pollen off the body with a dissecting needle (see Goldblatt et al., 1998a, b). The residue from needle probes was collected on glass slides and mounted in one or two drops of Calberla's fluid (Ogden et al., 1974). In the case of long-proboscid flies, which are large insects, sites of pollen deposition are often quite discrete for a particular plant visited, and pollen species can usually be identified without recourse to microscopic examination due to pollen color and position. Pollen grains were identified microscopically by comparison with a reference set of pollen grain preparations made from plants flowering at study sites. *Babiana* pollen grains are recognized by their large size, perforate-scabrate exine, and monosulcate aperture with prominent 2-banded operculum (Goldblatt et al., 1991).

Insect specimens were identified by R. W. Brooks, University of Kansas (Apoidea); H. Dombrow, Worms, Germany (Scarabaeidae); and J. C. Manning (Diptera, Lepidoptera). Voucher specimens are deposited at the Snow Entomological Museum, Lawrence, Kansas.

Results

INFLORESCENCE PHENOLOGY AND FLORAL LIFE SPAN

Babiana species are corm-bearing geophytes that have an erect or spreading inflorescence of sessile flowers (a spike) arranged in a spiral, in two ranks, or in more or less a single rank (Figs. 1A, 2A). Exceptionally, the spike may reach to 1 m or more in *B. thunbergii* Ker Gawl., but lengths of 10–40 cm are more usual and several species have the flowers borne close to the ground with the spike reaching only shortly above the ground. Aerial stems may be simple or branched, sometimes repeatedly, and the lateral branches bear fewer flowers than the main axis. Flowering is closely synchronized within the same population and generally lasts two to four weeks. *Babiana ringens* (L.) Ker Gawl. is unique in having the thickened, sturdy main axis sterile, and the flowers are borne on a lateral branch close to ground level. The sterile axis functions as a perch for visiting sunbirds (Marloth, 1915; Goldblatt et al., 1999). Flowering periods in the genus subdivide into a winter season (May to July) or a spring season (August to early October) in the southern African winter-rainfall zone (Table 2); only *B. bainesii* Baker, not included in our study, flowers in late summer and autumn (February to April) in eastern southern Africa, which has a summer-rainfall climate with a cold, dry winter.

The pattern of anthesis on an inflorescence is acropetal. In all species, a mature bud expands in the early morning, and, in most species, the flowers close again in the late afternoon or at sunset. Exceptions are members of section *Antholyzoides*; the two bird-pollinated species, *Babiana ringens* and *B. thunbergii*; and the putatively moth-pollinated *B. noctiflora* Goldblatt & J. C. Manning, *B. patersoniae* L. Bolus, and *B. virginea* Goldblatt. In the two bird-pollinated species, as well as in *B. spathacea* (L. f.) Ker Gawl., the tepal orientation is apparently too complex for the flowers to close, whereas in the moth-pollinated species the flowers remain open to permit access to pollinators. A flower typically lasts three to five days under greenhouse conditions when not pollinated but maintains the pattern of opening and closing at specific times. We have no data on longevity in cross-pollinated flowers. Flower buds on the same inflorescence open sequentially, usually one to two days apart. On plants with branched stems, there may be several flowers open at any time. When flowers close, the tepals loosely cloak the anthers and stigmas. Ambient temperature influences anthesis and on cold (less than 15°C), overcast, or misty days, a flower may not open at all, or the normal timing of opening may be delayed until conditions are favorable.

Flowers of all species studied are protandrous. The anthers dehisce longitudinally within one to four hours after the tepals first unfold. This depends to some extent on ambient temperature and humidity (see above), and anthers dehisce later in the day under wet-cool conditions. The style divides into three

Table 1. Study sites and voucher information for *Babiana* species studied. Vouchers are housed at MO (Goldblatt, Goldblatt & Manning) or at NBG (all collectors) unless indicated to the contrary. All study sites are in South Africa.

Species	Study site	Voucher
B. ambigua (Roem. & Schult.) G. J. Lewis	Western Cape, Mamre Veld Reserve	*Goldblatt & Porter 11878*
B. angustifolia Sweet		
site 1	Western Cape, Versfeld Reserve, Darling	*Goldblatt & Manning s.n.*, no voucher
site 2	Western Cape, Elandsberg Farm, Gouda	*Goldblatt 11221*
B. attenuata G. J. Lewis	Northern Cape, near Gargams	*Goldblatt & Manning 11323*
B. brachystachys (Baker) G. J. Lewis	Northern Cape, near Hondeklipbaai	*Goldblatt & Manning 9997*
B. carminea J. C. Manning & Goldblatt	Western Cape, Knersvlakte	*Goldblatt & Manning 10662*
B. cedarbergensis G. J. Lewis	Western Cape, Cedarberg Mtns.	*Goldblatt & Porter 12165*
B. crispa G. J. Lewis	Northern Cape, Bidouw Hills	*Goldblatt & Manning 11077*
B. cuneata J. C. Manning & Goldblatt	Western Cape, Katbakkies Pass	*Goldblatt & Manning 11457*
B. curviscapa G. J. Lewis		
site 1	Northern Cape, Leliefontein	*Goldblatt & Manning 10007*
site 2	Northern Cape, Spektakel Pass	*Goldblatt & Manning 9970*
site 3	Northern Cape, S of Springbok	*Goldblatt & Manning 11326*
site 4	Northern Cape, Kamiesberg, farm Botuin	*Goldblatt & Porter 11903*
site 5	Northern Cape, Springbok	*Goldblatt & Porter 12087*
B. dregei Baker		
site 1	Northern Cape, Kamiesberg, farm Sneeukop	*Goldblatt & Manning 9279*
site 2	Northern Cape, near Leliefontein	*Rodin 1445*
B. ecklonii Klatt		
site 1	Western Cape, Gifberg	*Goldblatt & Manning 10967*
site 2	Western Cape, Piekenierskloof Pass	*Goldblatt & Porter 12262*
B. engysiphon J. C. Manning & Goldblatt	Northern Cape, Moedverloor rd.	*Goldblatt & Porter 12137*
B. fimbriata (Klatt) Baker	Northern Cape, near Garies	*Goldblatt & Vänni 11132*
B. flabellifolia Klatt	Northern Cape, Anenous Pass	*Goldblatt & Manning 11367*
B. fragrans (Jacq.) Ker Gawl.		
site 1	Western Cape, Signal Hill, Cape Town	*Sidey 2186*
B. framesii L. Bolus		
site 1	Northern Cape, Nieuwoudtville Reserve	*Vordenstam 3012* (S)
site 2	Northern Cape, Farm Glenlyon	*Vänni 159*
B. geniculata G. J. Lewis	Western Cape, Biedouw Valley	*Goldblatt & Manning 9923*
B. inclinata Goldblatt & J. C. Manning		
site 1	Western Cape, near Porterville	*Goldblatt & Manning 9363*
site 2	Western Cape, N of Piketberg	*Goldblatt & Manning 11932*
B. latifolia L. Bolus	Western Cape, N of Piketberg	*Goldblatt & Porter 12127*
B. leipoldtii G. J. Lewis	Western Cape, Mamre Rd.	*Goldblatt & Nänni 11089*
B. lineolata Klatt		
site 1	Western Cape, Cold Bokkeveld	*Goldblatt 11631*
B. melanops Goldblatt & J. C. Manning		
site 1	Western Cape, near Mamre	*Goldblatt & Nänni 11095*
site 2	Western Cape, Darling Hills	*Goldblatt & Nänni 11095A*
site 3	Western Cape, S of Tulbagh	*Goldblatt 11420*
B. montana G. J. Lewis	Western Cape, Fairfield	*Goldblatt & Nänni 10250A*
B. mucronata subsp. *mucronata* (Jacq.) Ker Gawl.		
site 1	Western Cape, Olifants River valley	*Goldblatt & Manning 10937*
site 2	Western Cape, Piekeniers Kloof	*Goldblatt & Manning 11674*
B. nana (Andr.) Spreng.	Western Cape, Mamre	*Goldblatt & Nänni 11549*
B. noctiflora Goldblatt & J. C. Manning	Western Cape, Paardeberg	*Goldblatt & Nänni 11172*
B. odorata L. Bolus		
site 1	Western Cape, near Malmesbury	*Goldblatt 2184*
site 2	Western Cape, near Hopefield	*Goldblatt & Manning 11418*
B. papyracea Goldblatt & J. C. Manning	Northern Cape, Nieuwoudtville	*Goldblatt 11609, 11617*
B. patersoniae L. Bolus	Western Cape, near Napier	*Goldblatt & Manning 9954*

Table 1. Continued.

Species	Study site	Voucher
B. patula N. E. Br.	Western Cape, near Worcester	*Goldblatt 11091*
B. praemorsa Goldblatt & J. C. Manning	Northern Cape, Hantamsberg	*Goldblatt & Manning 9961*
B. pubescens (Lam.) G. J. Lewis	Northern Cape, Garies hills	*Goldblatt & Manning 9907*
B. purpurea (Jacq.) Ker Gawl.	Western Cape, near Botrivier	*Goldblatt 11112*
B. pygmaea (Burm. f.) Baker	Western Cape, near Hopefield	*Goldblatt & Manning 11415*
B. regia (G. J. Lewis) Goldblatt & J. C. Manning	Western Cape, near Kraaifontein, Farm Joostenbergkloof	*Goldblatt & Manning 11558*
B. rigidifolia Goldblatt & J. C. Manning	Western Cape, Botterkloof Pass	*Goldblatt & Manning 10523*
B. ringens (L.) Ker Gawl.		
site 1	Western Cape, near Yzerfontein	*Goldblatt s.n.*, no voucher
site 2	Western Cape, N of Aurora	*Goldblatt s.n.*, no voucher
B. rubrocyanea (Jacq.) Ker Gawl.	Western Cape, Darling, Waylands	*Goldblatt & Manning 11555*
B. sambucina subsp. *sambucina* (Jacq.) Ker Gawl.		
site 1	Western Cape, near Touwsrivier	*Goldblatt & Nänni 11465*
site 2	Western Cape, near Barrydale	*Goldblatt & Manning 9991*
site 3	Western Cape, near Ladismith	*Goldblatt & Porter 12184*
site 4	Western Cape, Kammanassie foothills	*Goldblatt & Porter 12289*
site 5	Western Cape, Cango valley	*Goldblatt & Porter 11838*
subsp. *longibracteata* G. J. Lewis	Northern Cape, near Nieuwoudtville	*Goldblatt & Manning 9973*
B. scabrifolia Brehm ex Klatt		
site 1	Western Cape, Olifants River valley	*Goldblatt & Manning 11081*
site 2	Western Cape, near Trawal	*Goldblatt & Manning 11445*
B. scariosa G. J. Lewis		
site 1	Northern Cape, S of Nieuwoudtville	*Goldblatt 11609*
site 2	Western Cape, Kobee valley	*Goldblatt 11614*
B. sinuata G. J. Lewis	Western Cape, near Bullshoek	*Goldblatt 11443*
B. spathacea (L. f.) Ker Gawl.		
site 1 (cream flowers)	Northern Cape, 19 km S of Nieuwoudtville	*Goldblatt & Manning 10033*
site 2 (lilac flowers)	Northern Cape, 8 km S of Nieuwoudtville	*Goldblatt & Manning 10034*
B. spiralis Baker	Northern Cape, Garies hills	*Goldblatt & Nänni 11453*
B. stricta (Aiton) Ker Gawl.		
site 1	Western Cape, Brandvlei hills	*Goldblatt & Manning 11129; Goldblatt & Porter 12172A*
site 2	Western Cape, near Swellendam	*Goldblatt 11434*
B. thunbergii Ker Gawl.	Western Cape, Lambert's Bay	*Hall 4539*
B. tritonioides G. J. Lewis	Northern Cape, Komaggas	*Goldblatt & Porter 12095*
B. tubiflora (Burm. f.) Ker Gawl.		
site 1	Western Cape, Rondeberg	*Goldblatt s.n.*, no voucher
site 2	Western Cape, Ganzekraal	*Goldblatt & Manning s.n.*, no voucher
B. unguiculata G. J. Lewis	Northern Cape, S of Nieuwoudtville	*Goldblatt & Porter 12431*
B. vanzijliae L. Bolus		
site 1	Northern Cape, Matjesfontein, S of Nieuwoudtville	*Goldblatt & Nänni 11107*
site 2	Northern Cape, Blompad, Nieuwoudtville	*Goldblatt & Nänni 11401*
B. villosa (Aiton) Ker Gawl.		
site 1	Western Cape, near Tulbagh	*Goldblatt & Porter 11426A*
site 2	Western Cape, Malmesbury	*Goldblatt 11424*
B. villosula (J. F. Gmel.) Ker Gawl. ex Steud.		
site 1	Western Cape, Mamre Rd.	*Goldblatt 11088*
site 2	Western Cape, Potsdam	*Manning s.n.*, no voucher
site 3	Western Cape, Silvermine	*Goldblatt 11312*
B. virginea Goldblatt	Northern Cape, near Ganagga Pass	*Goldblatt & Manning 10746*

Table 2. Floral characteristics of *Babiana* species arranged according to flower type. Features are for populations studied and may not reflect the variation in the entire species. Measurements of the perianth tube were taken from the base of the tube to the point at which the tepals separate. + = presence of small quantities; ++ = moderate to large quantities of nectar; tr = trace amount too little to measure volumetrically. Flower shapes follow the terminology of Faegri and van der Pijl (1979).

Species	Flower shape	Flower color	Tube, mm	Scent	Nectar	Flowering time
***B. ambigua* group (pollinated by large-bodied anthophorine bees)**						
B. ambigua	gullet	bluish with white	10–19	musky	++	Aug.–Sept.
B. attenuata	gullet	bluish with white	25–35	violet	++	Aug.
B. cedarbergensis	tube-gullet	blue-violet with white	16–18	light violet	++	Sept.
B. crispa	gullet	pale blue with white	14–20	musky	+	July–Aug.
B. cuneata	tube-salver	blue-violet with white	40–45	none	++	Aug.–Sept.
B. fimbriata	gullet	red-purple with yellow	10–12	sweet-clove	+	Aug.–Sept.
B. flabellifolia	gullet	blue or lilac with white	12–35	violet	+	July–Aug.
B. fragrans	gullet	violet or yellow with white	18–20	violet-clove	+	Aug.–Sept.
B. inclinata	gullet	blue with white	ca. 10	none	+	Sept.–Oct.
B. lineolata	gullet	blue with white	8–15	none	+	Sept.
B. montana	gullet	blue-mauve with white	15–20	light spicy	++	Aug.–Sept.
B. mucronata	gullet	blue-mauve with white	15–28	cinnamon-vanilla or musk	++	Aug.–Sept.
B. nana	gullet	bluish or pink with white	12–17	violet-rose	++	Aug.–Sept.
B. odorata	gullet	pale and deep yellow	10–14	violet, spice, and acrid musk	++	July–Aug.
B. patula	gullet	mauve and yellow	10–14	violet-rose	++	July–Aug.
B. purpurea	salver	pink to mauve, anthers blue-black	18–28	light rose	+	Aug.–Sept.
B. sambucina	salver	blue with white	35–60	strong violet	++	Aug.–Sept.
B. scabrifolia	gullet	blue with white	12–18	Narcissus	+	July–Aug.
B. scariosa	gullet	lilac-mauve with blue	12–20	none	++	Sept.
B. sinuata	gullet	blue with yellow	8–9	light spicy	+	Sept.
B. spiralis	gullet	blue or mauve-pink with yellow	8–10	sweet-vanilla	+	Aug.–Sept.
B. tritonioides	gullet	mauve with yellow	6–8	violet	+	Aug.
B. unguiculata	gullet	pale yellow with white	10–12	light spicy	+	Aug.–Sept.
***B. villosula* group (pollinated by pollen-collecting female bees)**						
B. leipoldtii	salver	blue, dark center, anthers white	15–20	light violet or acrid musk	+	Aug.
B. villosula	bowl-salver	blue-mauve, white center, anthers blue to mauve	18–30	musky-violet	tr	July–Aug.
***B. framesii* group (pollinated by long-proboscid flies)**						
B. brachystachys	tube-salver	pink with red	70–75	none	++	Sept.–Oct.
B. curviscapa	tube-gullet	blue-violet or magenta with white	36–43	none (magenta flowers) or light rose	++	Aug.–Sept.
B. dregei	tube-gullet	blue-violet with white	50–65	light rose	++	mainly Aug.
B. ecklonii	tube-gullet	violet with white	35–45	none	++	Sept.
B. engysiphon	tube-gullet	violet with white	35–47	none	++	Aug.–Sept.
B. framesii	tube-gullet	blue-purple with white	60–70	light violet	++	Aug.–Sept.
B. geniculata	tube-gullet	blue-purple with white	35–45	none	++	Aug.–Sept.
B. latifolia	tube-gullet	violet with white	30–40	none	++	Sept.
B. praemorsa	tube-salver	violet with white	40–60	none	++	Aug.–Sept.
B. pubescens	tube-gullet	blue-purple with white	ca. 50	none	++	Aug.–Sept.
B. rigidifolia	tube-gullet	violet with white	38–55	none	++	Sept.
B. sambucina subsp. *longibracteata*	tube-gullet	blue with white	40–55	violet or none	++	Aug.–Sept.
B. spathacea	tube-gullet	cream with red	35–45	none	++	Sept.–Oct.
B. tubiflora	tube-gullet	white with red	45–80	none	++	Aug.–Sept.

Table 2. Continued.

Species	Flower shape	Flower color	Tube, mm	Scent	Nectar	Flowering time
B. tubulosa	tube-gullet	white with red	65–90	none	++	Aug.–Sept.
B. vanzijliae	tube-gullet	yellow with cream	18–50	spicy musk	++	Aug.–Sept.
***B. pygmaea* group (pollinated partly or exclusively by hopliine beetles)**						
B. angustifolia	bowl	deep violet, anthers black	10–17	none	tr	mainly Sept.
B. melanops	salver	pale to dark blue-mauve, darker center, anthers blue-violet to black	16–27	lemon-violet or none	+ or none	Aug.–Sept.
B. papyracea	salver	blue	35–45	none	none	Oct.
B. pygmaea	bowl	yellow, brown or purple center	15–25	none	none	Aug.–early Sept.
B. regia	bowl	dark blue, red center, anthers rust-brown	10–12	none	tr	Sept.–Oct.
B. rubrocyanea	bowl	dark blue, red center, anthers rust-brown	15–20	none	+	Aug.–Sept.
B. stricta	salver	white, mauve or pink, lower lateral tepals often white or yellow	10–18	faint rose	+	Aug.–Sept.
B. villosa	salver	deep red to magenta or pink, anthers black	12–17 or 30–36	none	none	Aug.–Sept.
***B. patersoniae* group (pollinated by moths)**						
B. noctiflora	tube-gullet	cream, yellow markings	33–50	heavy sweet clove	++	Sept.
B. patersoniae	tube-gullet	cream flushed pink or blue	20–30	heavy sweet clove	++	Aug.–Oct.
B. virginea	tube-gullet	cream flushed mauve	60–65	heavy sweet clove	++	Sept.–Oct.
***B. ringens* group (pollinated by sunbirds)**						
B. carminea	tube-gullet	red with yellow marks	56–60	none	++	July–Aug.
B. ringens	gullet	red, yellow throat	32–45	none	++	Aug.–Sept.
B. thunbergii	gullet	red	30–40	none	++	July–Aug.

slender branches at a point between the mouth of the perianth tube and the apex of the exserted anthers, depending on species. The style branches, the adaxial surfaces of which comprise the stigmatic surfaces, are held erect when the flower first opens but diverge later during the same day, spreading outward above or between the erect or divergent anthers. The stigmatic surfaces typically appear to become receptive on the second day of flowering, when the stigma lobes expand and then appear minutely hairy and glistening under a 10× hand lens.

Open flowers are either bilaterally symmetric (zygomorphic) and then held suberect or face to the side (Figs. 1, 2, 3C), or are radially symmetric and held erect or suberect with the tepals slightly cupped or spreading horizontally from the base (Fig. 3A, B). The stamens are symmetrically disposed in radially symmetric flowers or are unilateral and arcuate, lying below or in front of the dorsal tepal in zygomorphic flowers. The filaments are inserted just above the apex of the narrow part of the tube and are exserted a short distance. Anthers are developmentally extrorse with loculicidal dehiscence, and the pollen adheres to the dehisced anther locules. An unusual feature of a few species of section *Babiana* is the sagittate anthers with an expanded connective, broadest at the base (Fig. 3B). The anthers and pollen in these species (e.g., *B. melanops* Goldblatt & J. C. Manning, *B. stricta* (Aiton) Ker Gawl., *B. villosa* (Aiton) Ker Gawl.) are darkly pigmented, either blackish or deep blue. In other species, the anthers and pollen are generally pale blue to whitish or rarely pale yellow.

FLORAL PRESENTATION AND POLLINATION SYSTEMS

Five main pollination systems can be recognized in *Babiana*, each characterized by a suite of morphological features including floral symmetry and orientation, tepal color, floral fragrance, and nectar quantity and concentration. Comparative floral traits of these systems are summarized in Table 2. The pollination systems and associated floral features are not always correlated with presumed natural relationships among the species, although these are still not completely understood (Goldblatt & Manning, 2007).

The Babiana ambigua *group.* In this group (Table 2), of which *Babiana ambigua* (Roem. & Schult.) G. J.

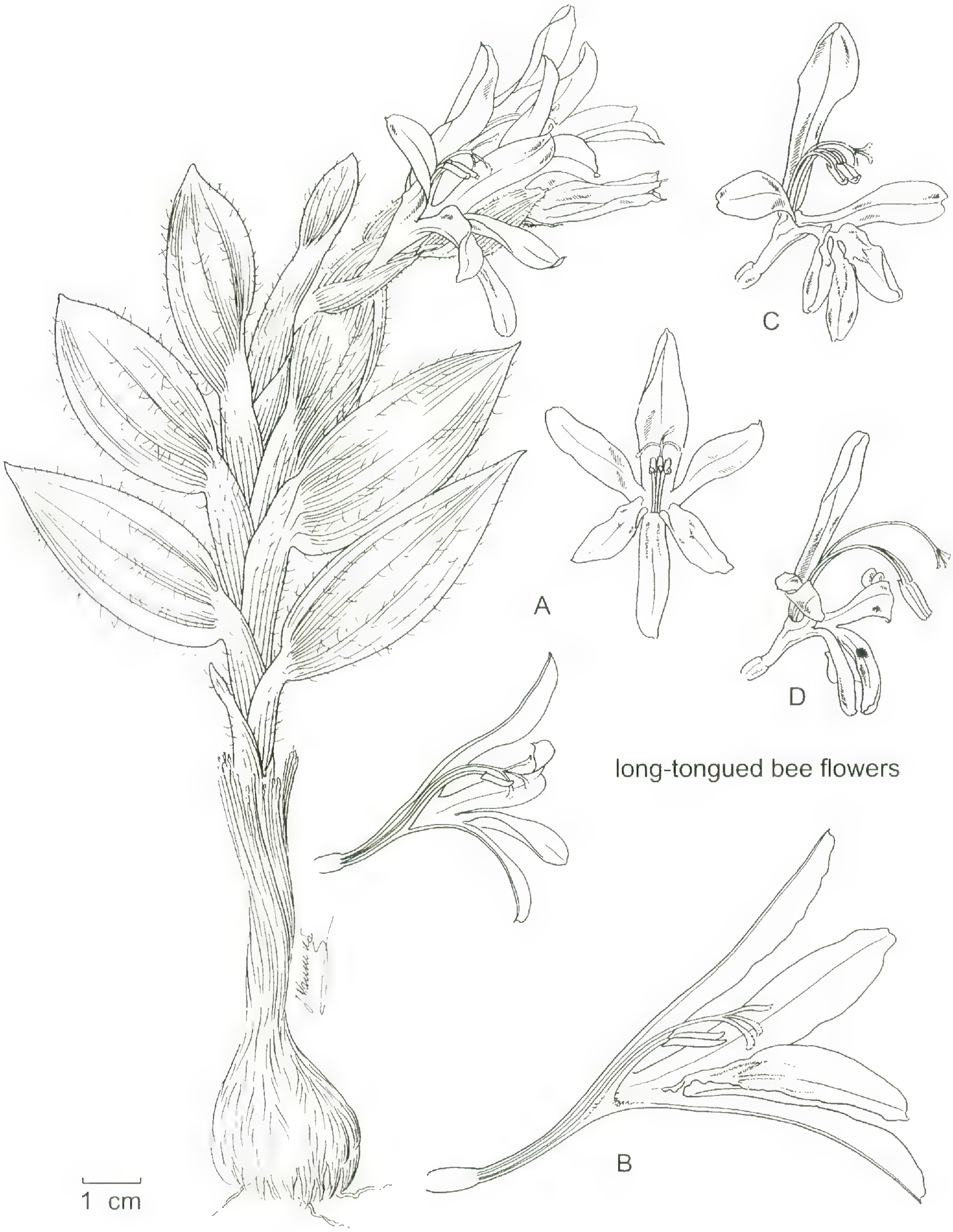

Figure 1. Flowers of *Babiana* species pollinated by large-bodied, long-tongued bees. —A. *Babiana rubella* (sect. *Teretifolieae*) with whole plant, and front and longitudinal section of flower. —B. *Babiana grandiflora* Goldblatt & J. C. Manning (sect. *Teretifolieae*), longitudinal section of flower. —C. *Babiana spiralis*, whole flower. —D. *Babiana sinuata* (both sect. *Exohebeoides*), whole flower; note strongly arched stamens and style. Drawn by John Manning from live plants. Scale bar = 1 cm; B, approximately 2× scale bar.

Lewis, *B. nana* (Andr.) Spreng., and *B. rubella* Goldblatt & J. C. Manning are typical examples, the flower is zygomorphic with a bilabiate perianth (Fig. 1A–D). Both style and stamens are unilateral and lie arched over the lower tepals, with the anthers facing the landing platform formed by the lower three tepals and their adaxial (sterile) surface lying just below the dorsal tepal. The style lies arched above the unilateral stamens and divides opposite to slightly beyond the anther apices, while the style branches are

3–6 mm long and arch outward. The receptive ends of the style branches, which comprise the stigmas, are thus held distant from the anthers and pollen (i.e., herkogamy). Anthers and pollen are typically cream, pale yellow, or pale blue, but *B. purpurea* (Jacq.) Ker Gawl. has blackish to dark blue pollen and the anthers have a wider connective that separates the anther lobes (thecae) on the abaxial (fertile) side by a narrow band of dark, sterile tissue. Flowers are scented, often strongly so, with the scent ranging from that of violets, roses, or freesias, to a spicy, acrid to somewhat metallic odor, or combinations of these.

The perianth is typically pale to deep blue or violet, or less often deep pink to purple (*Babiana purpurea*) or pale yellow (*B. odorata* L. Bolus, *B. noctiflora*). The lower lateral tepals have large, white median markings, often edged with dark blue, red, or purple (Table 2), and are held closely together, forming a landing platform. The perianth tube is funnel-shaped, curved to more or less straight, and typically ranges from 6–45 mm long, exceptionally to 60 mm in *B. sambucina* subsp. *sambucina* (Jacq.) Ker Gawl. The tube is divided into a narrow, cylindric lower part usually 6–12 mm long and an expanded funnel-shaped upper part that forms a widening throat ca. 5–8 mm long, which snugly accommodates the upper body of a large bee. The tube contains nectar, which may extend upward a short distance from the base. Occasionally, the lower part of the tube is elongated, but then the lower portion has thick walls and is narrow internally, with the interior blocked by the style so that nectar accumulates well above the tube base, the result of capillary movement. The small amount of nectar is thus forced into the upper half of the tube. The perianth tube may be shorter or longer than the tepals, markedly so in some forms of *B. bainesii* and *B. sambucina* subsp. *sambucina*. In these species, the thick walls of the tube tightly enclose the style and nectar is forced into the upper 10 mm of the slender part of the tube. Such tubes are characteristic of acaulescent species and may be envisaged as functioning as a pseudostem, thus raising the flower above the basal cluster of leaves where it may be more easily seen and visited by insects. This aspect of the perianth tube must be carefully examined before the pollination ecology of such species can be correctly understood.

Flowers of most species in this group are actively visited by apid bees (Table 3), either the native honeybee, *Apis mellifera*, or *Anthophora* species, most commonly *A. diversipes* and *A. rufidicaudis*. *Babiana inclinata* Goldblatt & J. C. Manning is also visited and evidently pollinated by the horsefly *Philoliche atricornis*, which has a proboscis 4–5 mm long, thus comparable with the probosces of apid bees.

Without exception, bees appear to be foraging for nectar and were rarely seen actively scraping pollen from the anthers. Visits are brief, less than two seconds per flower. As a bee forces its body into a flower and inserts its mouthparts down the narrow part of the perianth tube, the dorsal part of its head and thorax come into contact with the anthers or style branches. The dorsal part of the insect becomes covered with pollen, which is transported to other flowers. Despite this passive mode of pollen transfer, bees are often found to carry substantial loads of pollen in their scopae or corbiculae, which must be the result of grooming activity after pollen loads are swabbed on a bee's head and thorax. Bees that visit these *Babiana* flowers are polyphagic foragers (Table 3), and individuals were usually found to carry the pollen of various co-blooming species, most often *Bulbine* Wolf (Asphodelaceae), *Lachenalia* J. Jacq. (Hyacinthaceae), *Lobostemon* Lehm. (Boraginaceae), and other genera of the Iridaceae, both on various parts of their bodies and in their scopae or corbiculae. Curiously, despite this nectar-foraging pattern, all *Apis* individuals collected on *B. ambigua* at two study sites had corbiculae comprising pure loads of *Babiana* pollen.

Nectar characteristics of species belonging to this pollination group have relatively high sugar concentrations, ranging from a mean of 22% sucrose equivalents in *Babiana ambigua* and *B. crispa* G. J. Lewis to a maximum of more than 50% in *B. tritonioides* G. J. Lewis. Standing crop nectar volumes are relatively small, with a range measured in 22 species of 0.3–4.6 µl (Table 4) per flower and exceptionally 11.0 µl in one specimen of *B. sambucina* subsp. *sambucina*.

Visits to two members of the *Babiana ambigua* group, *B. cedarbergensis* G. J. Lewis and *B. spiralis* Baker, by the long-proboscid fly *Prosoeca peringueyi* (Table 3) merit mention. Flies actively visited these two species at our study sites but did not contact anthers or stigmatic tips of the style branches with their bodies. This is because the length of the perianth tube is shorter than their proboscis length (16–18 mm in *B. cedarbergensis* and 8–10 mm in *B. spiralis*), while captured flies at both sites had probosces exceeding 38 mm. This fly must be regarded as a nectar thief for these *Babiana* species. None of the flies captured at the *B. cedarbergensis* and *B. spiralis* study sites carried pollen of either species.

The Babiana villosula *group.* In a second group of bee-pollinated *Babiana* species, the flowers are actinomorphic and rotate, with spreading to widely cupped tepals, and the perianth is uniformly colored blue to mauve or pink (in *B. blanda* (L. Bolus) G. J.

Table 3. Pollen load analysis of captured insects on *Babiana* species. Coleoptera: Scarabaeidae: *Anisochelus* Serville, *Anisonyx* Serville, *Dichelus* Serville, *Lepisia* Serville, *Lepithrix* Serville, *Pachycnema* Serville, *Peritrichia* Burmeister. Hymenoptera: Apidae: *Amegilla* Friese, *Anthophora* Latreille, *Apis* L., *Pachymelus* Smith, *Xylocopa* Latreille; Halictidae: *Lasioglossum* Curtis. Diptera: Nemestrinidae: *Prosoeca* Schiner, *Moegistorhynchus* Wiedemann. Tabanidae: *Philoliche* Wiedemann. Lepidoptera: Noctuidae: *Syngrapha* Hubner. No sunbirds (*Nectarinia* Illiger) were captured and hence pollen loads were not analyzed.

Plant and insect taxon	No. of insects carrying pollen load(s): Host flower only	Host flower + other sp.
***B. ambigua* group**		
B. ambigua		
Apis mellifera L.	2	0
B. attenuata		
Anthophora diversipes Friese ♀	0	1
Anthophora krugeri Eardley & Brooks ♀	0	1
Amegilla sp. indet. ♀	0	1
Anthophora sp. indet. ♂	1	0
Pachymelus peringueyi Friese ♂	1	0
B. cedarbergensis		
Prosoeca peringueyi Lichtwardt*	0	0
B. fragrans		
Anisonyx ursus Fabricius*	0	4
Anthophora diversipes (not captured)		
B. inclinata site 1		
Philoliche atricornis Wiedemann site 2	5	0
Anthophora diversipes ♀*	0	1
Anthophora krugeri ♀	0	1
B. montana		
Apis mellifera	2	0
B. mucronata site 1		
Anthophora diversipes 2♀ 2♂ site 2	0	4
*Apis mellifera**	2	3
B. nana		
Anthophora diversipes ♀	0	2
B. odorata site 1		
Anthophora krugeri ♀	2	3
Apis mellifera	1	0
Anisonyx ditus Peringuey	0	0
B. patula		
Apis mellifera	1	2
Anthophora diversipes ♀	0	1
B. purpurea		
Anthophora rufidicaudis Cameron* ♀	0	2
B. sambucina subsp. *sambucina* site 1		
Anthophora diversipes ♀ site 2	0	1

Table 3. Continued.

Plant and insect taxon	No. of insects carrying pollen load(s): Host flower only	Host flower + other sp.
*Anthophora rufidicaudis** ♀ site 3	0	2
Anthophora flaviscopa Eardley* ♀ site 4	0	2
B. scabrifolia site 1		
*Apis mellifera** site 2	0	4
Apis mellifera	0	3
B. scariosa		
Anthophora abrochia Eardley Brooks* ♀	0	3
B. sinuata		
Apis mellifera	1	1
B. spiralis		
Pachymelus peringueyi ♀*	0	2
*Prosoeca peringueyi**	0	0
*Anthophora diversipes** ♀	0	1
B. tritonioides		
Anthophora flaviscopa ♀*	0	1
B. unguiculata		
Anthophora diversipes ♀*	0	2
Total	18	48
***B. villosula* group**		
B. villosula site 1		
Apis mellifera site 2	2	1
*Apis mellifera** site 3	3	0
*Apis mellifera**	4	0
Total	9	1
***B. framesii* group**		
B. curviscapa site 1		
*Prosoeca peringueyi** site 2	0	3
Prosoeca peringueyi site 4	0	2
*Prosoeca peringueyi** site 5	2	2
*Prosoeca peringueyi**	0	2
B. ecklonii		
*Prosoeca peringueyi**	0	2
B. engysiphon		
*Prosoeca peringueyi**	4	0
B. framesii site 1		
Prosoeca sp. indet. 1 site 2	2	3 (1 fly only with pollen of another species)
Prosoeca sp. indet. 1*	0	3
B. geniculata		
Prosoeca peringueyi	0	3
B. latifolia		
Prosoeca peringueyi	2	0
B. praemorsa		
Prosoeca sp. indet. 1	0	3
B. pubescens		
*Prosoeca peringueyi**	0	7
B. rigidifolia		

Table 3. Continued.

Plant and insect taxon	No. of insects carrying pollen load(s): Host flower only	Host flower + other sp.
Prosoeca peringueyi	0	2
B. sambucina subsp. *longibracteata**		
Prosoeca sp. indet. 1	0	3
B. spathacea		
Noctuid and other moths (none captured)		
B. tubiflora		
Moegistorhynchus longirostris Wiedemann	0	4
B. vanzijliae site 1		
Prosoeca sp. indet. 2	2	0
*Apis mellifera**	2	1
Anthophora schulzei Friese 1♀ 1♂ site 2	0	2
Prosoeca sp. indet. 1*	6	0
Total	20	42
***B. pygmaea* group**		
B. angustifolia site 1		
Philoliche atricornis site 2	0	2
Unidentified hopliine	2	5
B. melanops site 1		
Apis mellifera	5	1
Xylocopa rufitarsis Lepeletier 2♂	2	0
Anthophora diversipes ♀	0	2
Anisonyx ursus	4	1
Anisonyx ditus site 2	2	0
*Anisonyx ursus** site 3	8	2
Dichelus sp. indet.	12	5
Anisonyx ursus	3	0
B. papyracea		
Anisochelus inornatus Burmeister*	4	1
B. pygmaea		
Lepisia rupicola Serville	5	4
Lepisia cf. *lebisiii* Schein	0	3
B. regia		
Anisonyx ursus	0	8
Lasioglossum (*Ctenomia*) sp. indet.	0	1
B. rubrocyanea		
Anisonyx ursus	1	0
Lepisia rupicola (Fabricius)	0	2
Pachycnema crassipes (Fabricius)	0	1 (3 individuals with no pollen)
Apis mellifera	0	1
Lasioglossum (*Ctenomia*) sp. indet.	0	1
B. stricta site 1		
Apis mellifera	0	2
Anisonyx ursus	2	1

Table 3. Continued.

Plant and insect taxon	No. of insects carrying pollen load(s): Host flower only	Host flower + other sp.
Lepithrix ornatella Peringuey	6	3
B. villosa site 1		
Lepithrix ornatella	6	5
Peritrichia rufotibialis Schein site 2	0	4
Lepithrix fulvipes Thunberg	5	5
Anisonyx ditus	1	4
Total	68	64
***B. patersoniae* group**		
B. noctiflora		
Syngrapha circumflexa Linnaeus	0	0
***B. ringens* group**		
B. ringens site 1		
Nectarinia violacea Linnaeus	not captured (see Goldblatt et al., 1999)	
B. thunbergii		
Nectarinia fusca Vieillot	not captured (see Goldblatt et al., 1999)	
Grand total	98	155

* Represents a fraction of the total number observed but not collected.

Lewis, not included in our study) either with darker pigmentation toward the tepal bases or paler in the throat. Flowers are lightly scented (*B. leipoldtii* G. J. Lewis, *B. radiata* Goldblatt & J. C. Manning, *B. villosula* (J. F. Gmel.) Ker Gawl. ex Steud). The stamens are symmetrically arranged, with the anthers held erect and surrounding the central style. The flowers close at night and expand again the next morning. Only a small amount of nectar is held near the apex of the floral tube, which is narrowed toward the base with the walls tightly enclosing the style. Of the five species with this floral presentation, we have pollinator observations only for *B. villosula*. The species flowers in the late winter or early spring, often when there are few other plants in bloom. Flowers of all three populations studied were visited by honeybees, *Apis mellifera*, which usually foraged briefly for nectar and then scraped the anthers with their legs, and accumulated uniform loads of the whitish pollen in their corbiculae.

Table 4. Nectar properties of *Babiana* species that produce measurable amounts of nectar. SD = standard deviation; (n) = number or individuals sampled; Fru = fructose; Glu = glucose; Suc = sucrose; n/a = not assessed. Nectar sugar chemistry was analyzed by B.-E. van Wyk. Data for some species are from Manning and Goldblatt (1996) and Goldblatt and Manning (2000).

Species	Volume, µl (n)	Mean, % sugar (SD)	Fructose	Glucose	Sucrose	Mean sucrose/ Glu + Fru (n)
***B. ambigua* group**						
B. ambigua	1.2–1.8 (7)	22.0 (4.0)	n/a	n/a	n/a	n/a
B. attenuata	1.7–2.4 (10)	28.1 (2.2)	n/a	n/a	n/a	n/a
B. cedarbergensis	0.8–1.6 (8)	28.4 (3.0)	n/a	n/a	n/a	n/a
B. crispa	2.8 (1)	22.0	n/a	n/a	n/a	n/a
B. cuneata	1.6–3.4 (10)	28.7 (3.9)	n/a	n/a	n/a	n/a
B. fimbriata	1.7–2.8 (10)	40.1 (4.2)	n/a	n/a	n/a	n/a
B. flabellifolia	0.9–1.8 (2)	25.5 (0.7)	n/a	n/a	n/a	n/a
B. fragrans	1.3–2.7 (10)	35.2 (3.4)	n/a	n/a	n/a	n/a
B. inclinata	0.8–2.8 (10)	36.6 (4.1)	n/a	n/a	n/a	n/a
B. lineolata	0.3–1.6 (7)	39.0 (8.4)	n/a	n/a	n/a	n/a
B. mucronata site 1	1.8–2.2 (3)	30.3 (2.9)	9	16	75	3.0 (1)
site 2	1.4–1.7 (4)	33.5 (3.0)	n/a	n/a	n/a	n/a
B. nana	1.7–2.2 (3)	36.3 (3.8)	n/a	n/a	n/a	n/a
B. odorata site 1	1.0–2.7 (5)	27.4 (5.7)	11	17	72	2.57 (1)
site 2	1.3–2.4 (4)	29.6 (3.1)	n/a	n/a	n/a	n/a
B. patula	1.3 2.1 (10)	42.3 (3.4)	n/a	n/a	n/a	n/a
B. purpurea	0.8–1.9 (10)	32.3 (1.6)	n/a	n/a	n/a	n/a
B. sambucina subsp. *sambucina* site 1	4.4–11.0 (2)	26.5 (7.8)	3	8	89	8.09 (1)
site 2	3.8–4.6 (10)	28.3 (1.1)	n/a	n/a	n/a	n/a
site 3	1.2–3.2 (10)	28.6 (2.3)	n/a	n/a	n/a	n/a
B. scabrifolia site 1	1.2–2.5 (7)	28.0 (4.3)	n/a	n/a	n/a	n/a
site 2	1.2–1.8 (10)	27.9 (3.1)	n/a	n/a	n/a	n/a
B. scariosa site 1	0.4–1.2 (6)	37.0 (5.6)	n/a	n/a	n/a	n/a
site 2	0.6–1.4 (5)	39.4 (4.5)	n/a	n/a	n/a	n/a
B. sinuata	1.5 2.4 (10)	27.8 (2.6)	n/a	n/a	n/a	n/a
B. spiralis	2.4–3.6 (10)	33.3 (3.7)	n/a	n/a	n/a	n/a
B. tritonioides	0.4–0.6	> 50	n/a	n/a	n/a	n/a
B. unguiculata	0.3–1.9 (5)	31.3 (4.1)	n/a	n/a	n/a	n/a
***B. villosula* group**						
B. leipoldtii	0.6–1.4 (10)	24.2 (1.3)	n/a	n/a	n/a	n/a
B. villosula	< 0.2 (10)	n/a	n/a	n/a	n/a	n/a
***B. framesii* group**						
B. brachystachys	5.3–8.2 (5)	23.4 (1.2)	8–19	24–27	54–58	1.28 (2)
B. curviscapa site 1	4.2–5.9 (3)	28.0 (2.0)	9–12	14–19	58–73	1.78 (4)
site 2	2.0–4.4 (5)	25.0 (n/a)	12	19	69	2.23 (1)
site 3	2.7–6.2 (5)	28.3 (1.2)	n/a	n/a	n/a	n/a
site 4	4.7–2.1 (8)	29.8 (2.3)	n/a	n/a	n/a	n/a
B. dregei site 1	3.9–9.6 (5)	22.0 (2.2)	13–15	19–21	64–68	1.94 (2)
site 2	1.8–4.1 (4)	23.3 (2.2)	n/a	n/a	n/a	n/a
B. ecklonii site 1	4.3–8.9 (5)	27.7 (2.0)	5–11	10–18	72–85	3.29 (3)
site 2	2.7–3.5 (3)	31.7 (1.5)	n/a	n/a	n/a	n/a
B. engysiphon	1.6–4.3 (7)	29.3 (3.1)	n/a	n/a	n/a	n/a
B. framesii site 1	2.6–6.4 (5)	25.5 (0.9)	7–9	12-14	77–81	3.84 (3)
site 2	2.6–3.3 (10)	28.3 (2.2)	n/a	n/a	n/a	n/a
B. geniculata	3.2–4.8 (5)	29.3 (1.2)	17	21	62	1.63 (1)
B. latifolia	1.8 8.8 (10)	29.9 (2.6)	n/a	n/a	n/a	n/a
B. praemorsa	3.9–9.6 (5)	27.0 (1.6)	3–7	10–14	75–79	4.53 (2)
B. pubescens	3.2–4.8 (5)	28.0 (1.7)	3–8	9–14	78–88	2.18 (2)
B. sambucina subsp. *longibracteata*	3.9–6.6 (3)	30.0 (n/a)	6 12	10–19	69–84	3.48 (3)

Table 4. Continued.

Species	Volume, µl (n)	Mean. % sugar (SD)	Fructose	Glucose	Sucrose	Mean sucrose/ Glu + Fru (n)
B. spathacea site 1	4.3–5.5 (3)	29.3 (1.1)	13–16	16–18	66–71	2.17 (2)
site 2	2.8–4.1 (10)	32.3 (2.6)	16–17	20	63–64	1.74 (2)
B. tubiflora site 1	2.3–5.0 (5)	25.6 (1.1)	9–15	14–22	63–77	2.57 (3)
site 2	4.5–6.2 (10)	24.9 (1.3)	n/a	n/a	n/a	n/a
B. vanzijliae site 1	1.7–2.2 (10)	28.7 (1.8)	12–20	15–17	63–73	2.13 (2)
***B. pygmaea* group**						
B. angustifolia	not detectable					
B. melanops site 1	0.8–1.8 (10)	24.2 (1.3)	n/a	n/a	n/a	n/a
site 2	not detectable					
site 3	not detectable					
B. pygmaea	not detectable					
B. regia	trace					
B. rubrocyanea	trace					
site 2	0.6–1.4 (10)	33.9 (2.9)	n/a	n/a	n/a	n/a
B. stricta site 1	trace					
B. villosa	trace or none evidently produced					
***B. patersoniae* group**						
B. noctiflora	4.8–6.2 (10)	30.6 (3.5)	n/a	n/a	n/a	n/a
B. patersoniae	1.7–2.8 (5)	31.7 (1.6)	2–5	4–9	86–94	8.34 (3)
B. virginea	17–22 (3)	26.0 (1.0)	n/a	n/a	n/a	n/a
***B. ringens* group**						
B. carminea	21.0–26.0 (3)	21.0 (2.6)	n/a	n/a	n/a	n/a
B. ringens site 1	19.5–23.4 (2)	23.0	9	20	71	2.45 (1)
site 2	25.0–28.0 (2)	23.5 (0.7)	12–13	17	70–71	2.39 (2)
B. thunbergii	24.0–30.0 (10)	25.5 (0.8)	15–21	18–25	60–65	1.65 (4)

Nectar of this pollination group has relatively modest sugar concentrations, with a mean of 24.2% sucrose equivalents in *Babiana leipoldtii*, the only species in the group for which we have data (Table 4). Standing crop nectar volumes are relatively small, with 0.6–1.4 µl measured in *B. leipoldtii*, or negligible.

The Babiana framesii *group.* In a third group. the flowers are zygomorphic in 17 species and actinomorphic in one more. Zygomorphic flowers have either a bilabiate perianth with the dorsal tepal erect and the lower tepals forming a platform, or the tepals spread equally with the dorsal tepal being somewhat larger and held apart from the others (Fig. 2A–E). All three lower tepals or just the lower laterals have pale contrasting markings. As in the *Babiana ambigua* group, the style and stamens are unilateral and lie arched over the lower tepals, with the anthers facing the landing platform. The style divides opposite to or slightly beyond the anther apices, and the style branches arch outward. The stigmatic ends of the style branches are thus held away from the anthers and pollen. Most distinctive of this group, the lower part of the perianth tube is elongated, usually hollow to the base, typically 35–70 mm long, or exceptionally to 80 or 90 mm in *B. tubiflora* (Burm. f.) Ker Gawl. and *B. tubulosa* (Burm. f.) Ker Gawl. The flowers are usually scentless (Table 2) or rarely strongly scented (*B. sambucina* subsp. *longibracteata* G. J. Lewis, *B. dregei* Baker, *B. vanzijliae* L. Bolus). As in the *B. ambigua* group, the flowers close at night and expand again the next morning. Flowers of the group have three quite different pigmentation patterns that are closely correlated with the insects that pollinate them.

The flowers produce relatively large amounts of sucrose-dominant nectar (Table 4). Volumes measured in 14 species range from 1.6 µl in *Babiana engysiphon* J. C. Manning & Goldblatt to 9.6 µl per flower in populations of *B. dregei* and *B. praemorsa* Goldblatt & J. C. Manning. Nectar sugar concentrations are relatively high, ranging from a mean of 22.0% sucrose equivalents in *B. dregei* to maxima of 30.0% in *B. sambucina* subsp. *longibracteata* and 32.3% in *B. spathacea*.

The perianth is usually predominantly blue to violet (Table 2), intensely so in *Babiana praemorsa*, and exceptionally red-purple in northern Namaqualand populations of *B. curviscapa* G. J. Lewis, and the lower tepals are marked with white, longitudinal diamond- to spear-shaped marks edged proximally in darker blue, purple, or red.

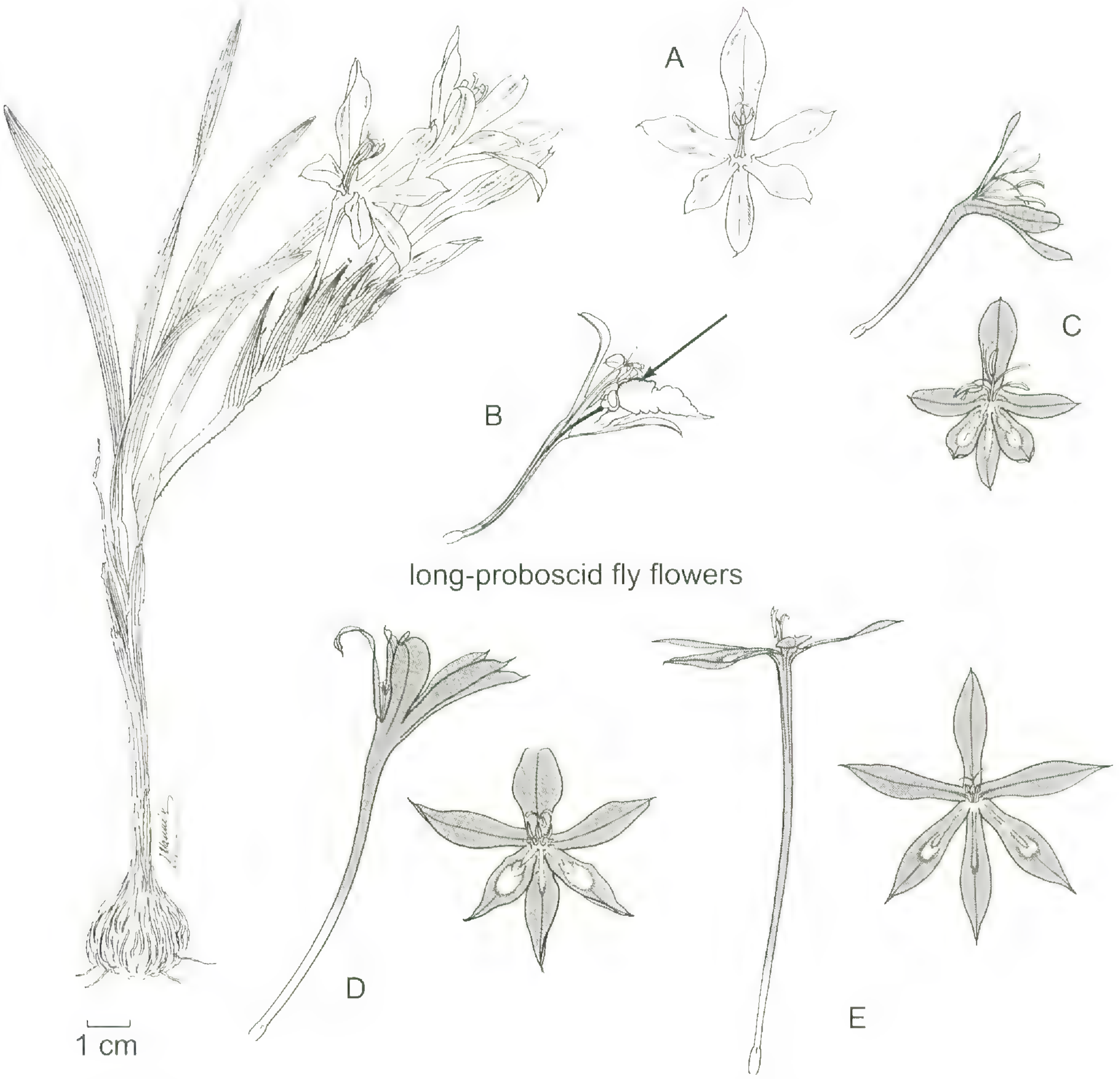

Figure 2. Flowers of *Babiana* species pollinated by long-proboscid flies. —A. Plant of *B. engysiphon*, showing long-tubed flowers, and front view of flower. —B. *Babiana curviscapa*, longitudinal section of flower showing orientation of *Prosoeca* fly when foraging for nectar; arrow indicates part of the fly's body that will contact anthers or style branches. —C–E. Lateral and front view of flowers of (C) *B. geniculata*, (D) *B. framesii*, (E) *B. praemorsa*, with shading for violet coloration, lower lateral tepals bearing pale markings (putative nectar guides). Drawn by John Manning from live plants. Scale bar = 1 cm; D and E, approximately 2× scale bar.

In a second species cluster in the *Babiana framesii* L. Bolus group, which includes the common West Coast *B. tubiflora*, as well as *B. tubulosa* and the rare Namaqualand *B. brachystachys* (Baker) G. J. Lewis, the perianth is white, often flushed pink on the outside, with dark pink to red markings on the lower tepals. We include *B. spathacea* in this group, noting that no long-proboscid fly has ever been collected over its range in the Western Karoo (Goldblatt & Manning, 2007). The only insects captured on the species are moths (Table 3), but we consider these opportunistic vistors, as moth flowers are always scented and often close during the day. It is possible the legitimate fly pollinator of *B. spathacea* is extinct.

Exceptionally, *Babiana vanzijliae* usually has a predominantly yellow perianth, sometimes flushed light mauve outside or on fading. We include it in the *B. framesii* group with some hesitation, not only because of the predominant yellow perianth color, but because the flowers are scented and the perianth tube is sometimes intermediate in length between those of typical members of the *B. ambigua* and *B. framesii* groups, ranging from 25–35 mm long, the narrow lower part 15–25 mm long.

The length of the perianth tube in some species in this group does not always reflect the length of the mouthparts of legitimate insect vistors. In *Babiana dregei*, the tube may be 50–65 mm long, but the lower portion of the tube has thick, fleshy walls and a narrow interior that is filled by the style (Manning & Goldblatt, 1996). Nectar is thus forced higher up the tube, where it is available to insect visitors with

Figure 3. Flowers of *Babiana* species pollinated by (A, B) hopliine beetles and (C) moths. —A. Plant of *B. papyracea* and top view of the radially symmetric flower. —B. *Babiana melanops*, with portion of the inflorescence and a single flower. —C. *Babiana noctiflora*, with inflorescence and side view of flowers showing the elongate perianth tube. Drawn by John Manning from live plants. Scale bar = 1 cm.

mouthparts exceeding 30 mm in length, less than the total length of the tube.

Most species in this group are pollinated exclusively by long-proboscid flies of the family Nemestrinidae. As described in *Lapeirousia* (Goldblatt et al., 1995) and *Sparaxis* (Goldblatt et al., 2000a), flowers in western southern Africa with long floral tubes and vivid, blue to purple flowers with white nectar guides are likely to be pollinated by one of two species of the genus *Prosoeca*, *P. peringueyi* or *Prosoeca* sp. 1, and this is the case in *Babiana*. Four species of the genus have already been reported as being pollinated by one of these two fly

species (Manning & Goldblatt, 1996; Goldblatt & Manning, 2000), and we add eight more to the list here.

We infer that the few species with white flowers with pink or red markings are adapted for pollination by a third nemestrinid fly, *Moegistorhynchus longirostris*, as are similarly proportioned and pigmented flowers in *Lapeirousia* (Goldblatt et al., 1995; Manning & Goldblatt, 1997) and *Gladiolus* (Goldblatt & Manning, 1999). This fly is restricted to the west coast and near interior of southern Africa, and this corresponds exactly to the range of the white- to pink-flowered, long-tubed species of *Babiana*. Our only observation for this cluster of species is for *B. tubiflora*, published elsewhere as *B. tubulosa* var. *tubiflora* (L. f.) G. J. Lewis (Manning & Goldblatt, 1997).

Both *Prosoeca* and *Moegistorhynchus* are polylectic foragers (Manning & Goldblatt, 1996, 1997; Goldblatt & Manning, 2000; Johnson & Steiner, 1997), and individuals were found to carry the pollen of co-blooming species with similar floral morphology, including *Pelargonium* L'Hér. ex Aiton (Geraniaceae), *Geissorhiza* Ker Gawl., *Gladiolus*, and *Lapeirousia* (all Iridaceae), and *Disa* P. J. Bergius (Orchidaceae) (Table 3). The long-tubed species of *Babiana* thus belong to one of two discrete guilds of plant species that share a similar floral appearance, the *Prosoeca peringueyi* or *Moegistorhynchus* pollination guilds as defined by Manning and Goldblatt (1996, 1997). The elongated proboscses of these flies are typically slightly longer than the length of the perianth tubes (or at least the hollow portion of the tube) of the plant species that they visit, but the flies are the only insects that are able to forage on the nectar held in the lower part of the perianth tube.

As we have described in detail elsewhere (Goldblatt & Manning, 1999, 2000), flies grasp the tepals with their tarsi and probe for nectar while continuing to vibrate their wings. As they force their bodies into the flower, pollen is passively deposited on specific parts of their bodies, in the case of *Babiana* species usually in the dorsal part of the thorax (Fig. 2B). In *B. geniculata* G. J. Lewis, in which the anthers are held well apart from one another (Fig. 2C), pollen deposition may be on the dorsal or lateral parts of the thorax (Manning & Goldblatt, 1996). Visual observation and pollen load analysis show that all fly species visit flowers of a range of other species during foraging bouts, most of which have morphologically convergent flowers that may be regarded as belonging to the same pollination guilds (Manning & Goldblatt, 1996).

Thus, *Prosoeca peringueyi* visits dark red, purple, or dark blue flowers of *Pelargonium crassicaule* L'Hér., *P. magenteum* J. J. A. van der Walt, *P. sericifolium* J. J. A. van der Walt, and species of Iridaceae including *Hesperantha latifolia* (Klatt) M. P. de Vos, *Lapeirousia jacquinii* N. E. Br., and *L. silenoides* (Jacq.) Ker Gawl., as well as co-blooming *Babiana curviscapa*, *B. dregei*, *B. ecklonii* Klatt, *B. engysiphon*, *B. framesii*, *B. geniculata*, *B. pubescens* (Lam.) G. J. Lewis, and *B. rigidifolia* Goldblatt & J. C. Manning, at sites in Namaqualand and Western Cape. A second fly, *Prosoeca* sp. 1, visits the red, purple, or violet flowers of species of Iridaceae including *H. oligantha* (Diels) Goldblatt, *L. oreogena* Schltr. ex Goldblatt, *L. jacquinii*, and *Romulea hantamensis* (Diels) Goldblatt, as well as co-blooming *B. praemorsa* (published as *B. flabellifolia* Klatt), *B. framesii*, and *B. sambucina* subsp. *longibracteata* (Manning & Goldblatt, 1996), at sites on the Bokkeveld Escarpment and Hantam Mountains. Likewise, along the west coast of Western Cape, *Moegistorhynchus longirostris* visits white, cream, or pink flowers of *P. longicaule* Jacq., *Disa draconis* (L. f.) Sw. (Orchidaceae), and several other Iridaceae including *Geissorhiza exscapa* (Thunb.) Goldblatt, *Gladiolus angustus* L., *L. anceps* (L. f.) Ker Gawl., and *Ixia paniculata* D. Delaroche, as well as *B. tubulosa* (Manning & Goldblatt, 1997; Goldblatt & Manning, 2000).

Exceptional for the *Babiana framesii* group in pigmentation and floral odor, *B. vanzijliae* has predominantly yellow flowers that, to the human nose, have a strong odor that represents a combination of violets and an unpleasant, acrid, insecticide-like component, for convenience here called spicy musk (e.g., Table 2). The flowers do, however, produce ample quantities of nectar, retained in the lower part of the tube and accessible only to two captured visitors, *Prosoeca* sp. 1 and a second undescribed *Prosoeca* species (*Prosoeca* sp. 2) with a proboscis 12–14 mm long (tube length at this study site was 30–35 mm). The flowers were also visited at this site by *Apis mellifera* workers and *Anthophora schulzei* (Table 3). Both bees attempted unsuccessfully to forage for nectar, but in doing so became covered in the yellow pollen of *B. vanzijliae*, thus accomplishing pollen transfer in the process. For these bees, the flowers of *B. vanzijliae* appear to function as mimics of the flowers of other species from which they do obtain nectar. *Prosoeca* sp. 1, which forages more frequently on the long-tubed purple flowers of *B. framesii*, *B. sambucina* subsp. *longibracteata*, *Lapeirousia jacquinii*, and *L. oreogena* that grow nearby, successfully pollinated *B. vanzijliae* with longer-than-usual perianth tubes at a second study site. Here, flowers had perianth tubes 35–50 mm long, while flies had proboscses 31–44 mm long (mean 36.9 mm, standard deviation 4.4, n = 15). Plants with shorter perianth tubes that were visited by flies with longer-than-average-length proboscses, however, were not pollinat-

ed by visiting flies. Their probosces were so long that when inserted to their full extent into the tube, their bodies did not contact either anthers or stigmas. Variation in the perianth tube of *B. vanzijliae* is notable. While plants at study site 1 have a tube 30–35 mm long, plants at our second study site had a tube 35–50 mm long. We suspect that the perianth tube length in flowers of *B. vanzijliae* may be tracking the proboscis length of local pollinators.

Visits to two members of the *Babiana ambigua* group, *B. cedarbergensis* and *B. spiralis*, by the long-proboscid fly *Prosoeca peringueyi* (Table 3) are discussed above under the *B. ambigua* group. We regard them as nectar thieves of these two species.

The Babiana pygmaea *group.* A fourth pollination group includes *Babiana melanops*, *B. pygmaea* (Burm. f.) Baker, *B. regia* (G. J. Lewis) Goldblatt & J. C. Manning, *B. rubrocyanea* (Jacq.) Ker Gawl., *B. stricta*, *B. villosa*, and a few more (Table 2) that have a salver- or bowl-shaped, radially symmetric perianth, sometimes marked with a contrasting, usually darker center, or possessing purple-black anthers with an expanded connective (Fig. 3A, B). These flowers may have a relatively short tube, 10–20 mm long, or an elongate tube up to 45 mm long, either containing only a trace of nectar near the apex or evidently no nectar. In species with longer tubes, the tube is filled internally by the style, which is closely sheathed by the thick walls of the tube. Nectar, when present, is thus restricted to the upper part of the tube. The tepals, up to 25 mm long, spread horizontally or arch slightly upward to form a shallow bowl. These flowers close in the late afternoon, and the tepals then cover the stamens and style branches. The tepals unfold again during late morning in warm weather. Perianth color is brilliant red to purple in *B. villosa*, bluish to violet with a blue-black center in *B. melanops*, or deep blue with a bright red center in *B. regia* and *B. rubrocyanea*. *Babiana pygmaea* has a large yellow perianth with a brownish to dull purple center. *Babiana stricta* is exceptional here in comprising populations with pinkish purple, blue-violet, or creamy white flowers, and, while the perianth is radially symmetric, the lower tepals have obscure darker markings toward the base. In *B. stricta* and *B. rubrocyanea* (Table 4), the flowers also produce measurable amounts of nectar.

The style is central and erect and surrounded by symmetrically disposed stamens in most of these species, but in *Babiana rubrocyanea* and *B. stricta* the stamens are unilateral and held to one side of the flower. Style branches are sometimes slender (as in most species of the genus), but the tips are enlarged and prominent in *B. rubrocyanea*, *B. villosa*, and often in *B. melanops*. The style divides below the base of the anthers or opposite the base to the middle of the anthers with the style branches then extending between the filaments or anthers. Species typically have dark-colored anthers and pollen, usually blackish or dark blue or, in *B. regia* and *B. rubrocyanea*, reddish brown. *Babiana melanops*, *B. stricta*, and *B. villosa* have large anthers, the connective of which is expanded so that the anther thecae are widely separated from one another. Flowers are usually unscented, but those of *B. stricta* are lightly sweet scented and in the southernmost population of *B. melanops* flowers have a light, pleasant odor of violets. The flowers of this population are the only ones in the species that also produce nectar in measurable quantities (Table 4).

Species of this group are visited predominantly by hopliine scarab beetles (Scarabaeidae: Rutelinae: Holpiini). *Babiana rubrocyanea* is visited by two beetle species, *Pachycnema crassipes* and *Anisonyx ursus*, and *B. melanops* is visited by a range of hopliine beetles depending on locality (Table 3), including *A. ursus* alone, or together with *A. ditus*, but the nectar-producing population is visited by a range of bees foraging for nectar as well as by occasional hopliines. *Babiana pygmaea* is visited by *Lepisia rupicola* and *Lepisia* cf. *lebisii*, while *B. regia* was visited at our study site by *Anisonyx ursus*.

The flowers of the *Babiana pygmaea* group have the stereotypical adaptations for the hopliine beetle pollination system described by Steiner (1998) and Goldblatt et al. (1998b): a large, bowl- or salver-shaped flower, constricted perianth tube containing little or no nectar, and bold, contrasting colors. The so-called beetle marks may be represented by different bands or blotches of color on the tepals or by enlarged dark anthers (*B. melanops*, *B. villosa*). *Anisonyx*, *Lepisia*, and *Pachycnema* species, and other hopliines spend between 12 seconds and 10 minutes (or longer) in a flower, either crawling about or at rest with their head directed toward the center of the perianth. Some visits last throughout the night as beetles take refuge in the closed flower until the next day.

Beetles captured on the flowers of these plants are invariably covered with the reddish brown or black pollen of the *Babiana* species and sometimes also pollen of some co-blooming taxa. Hopliine beetles use the flowers as sites for assembly, intraspecific competition, and mating, and although they feed on pollen and sometimes other floral tissue, they do little or no damage to flowers (Steiner, 1998; Goldblatt et al., 1998b).

The open perianth of species of the *Babiana pygmaea* group may also be visited by bees, and we have noted occasional visits to *B. regia* and *B. rubrocyanea* by unnamed species of *Lasioglossum*

(*Ctenomia*) (Halictidae) (Table 3). *Babiana stricta* appears to use both bees and hopliine scarab beetles equally, and while the spreading tepals that are held more or less horizontally seem well-suited to the preferences of beetles, the fragrance and presence of nectar provide an attractant and reward more typical of bee-pollinated flowers.

The Babiana patersoniae *group.* A small group of species, including *Babiana noctiflora*, *B. patersoniae*, and *B. virginea*, have intensely scented, white or cream to pale yellow or pale blue flowers, sometimes with light purple or pinkish markings on the lower tepals, and a long, cylindric, or weakly tapering perianth tube, 33–50 mm long in *B. noctiflora* (Fig. 3C) and 60–65 mm in *B. virginea* (Table 2). The flowers are unusual in the genus in remaining open at night, and all produce a complex, sweet-spicy odor. Flowers produce ample amounts of nectar, up to 6.2 µl in *B. noctiflora*, with relatively high sugar concentration (mean 26.0–31.7 sucrose equivalents) (Table 4). In the one species of the group examined for pollinators, *B. noctiflora*, the flowers are visited by long-proboscid moths. The single species of moth captured, *Syngrapha circumflexa* (Noctuidae), has a proboscis 16–17 mm long. Moths are active after dark and crawl over the flowers on an inflorescence, accumulating pollen deposited from the open flowers of each branch on which they alight.

The Babiana ringens *group.* Two species of the Namaqualand coast and Western Cape Province, *Babiana ringens* and *B. thunbergii*, have morphologically complex flowers with a bright scarlet-red perianth, strongly unequal tepals, and an elongate, sharply curved perianth tube, narrow in the lower half, abruptly expanded in the middle, and wide and cylindric above. The flowers are borne on horizontal branches and face away from the main stem, either well above ground in *B. thunbergii* or at ground level in *B. ringens*. In *B. ringens*, the main stem is represented by a stout, sterile stalk. The dorsal tepal is large and erect, with the margins curved inward partly enclosing the stamens and style, while the upper lateral and lower tepals spread outward. The style and stamens are unilateral and curved upward to lie close to the dorsal tepal, which partly encloses the unusually rigid filaments and style, both of which are dark red. The anthers are well-exserted and dark blue-black, and the elongate style divides well above the exserted anthers. The flowers are unscented and secrete large quantities of nectar, up to 30 µl in our study population of *B. thunbergii*. Mean nectar concentrations range from 23.0%–25.5% sucrose equivalents in these two species (Table 4).

Flowers of these two species are visited exclusively by sunbirds, *Nectarinia* species. Birds perch on the branches in *Babiana thunbergii* and either on the sterile main axis in *B. ringens* or sometimes on the ground; they push their bills into the upper part of the perianth tube and, evidently, take nectar from the narrow part of the tube with their tongues. We did not capture any sunbirds and so cannot say whether they carried pollen or not. Their foraging, however, invariably results in their head or neck contacting the anthers and style branches. This is clearly evident in published photographs of *N. famosa* visiting both *B. thunbergii* and *B. ringens* in the wild (Cowling & Pierce, 1999: 129; Manning, 2004: 115). Because sunbirds are the only animal visitor to these flowers, they must be assumed to be their sole pollinators.

We include the southern Namaqualand species, *Babiana carminea* J. C. Manning & Goldblatt in the *B. ringens* group, although it is clearly taxonomically distantly related to the other two members of the group and has an acaulescent growth habit. Like these two species, the flowers are red and have an elongate tube with the upper part relatively wide. We have no pollinator observations, but the flowers produce large amounts of nectar of relatively low sugar concentration, a feature that is also consistent with sunbird pollination.

NECTAR

Nectar glands are septal, as they are in the entire subfamily Crocoideae (Goldblatt, 1990; Rudall et al., 2003). Nectar is secreted from three minute pores at the top of the ovary (one per chamber) directly into the base of the perianth tube, where accumulated fluid is retained until removed by a foraging insect. In several species, the lower part of the tube may be extremely narrow internally, the walls thus tightly enveloping the style (see discussion above) so that nectar is forced by capillary action higher up the tube, sometimes to within a short distance of the tube mouth. Notable examples are acaulescent species including the long-tubed *Babiana attenuata* G. J. Lewis, *B. dregei*, forms of *B. bainesii*, and *B. sambucina*. Effective tube length for foraging insects is much less than the total tube length because, at least in *B. attenuata*, *B. bainesii*, and *B. sambucina*, nectar lies within 10 mm of the base of the wider part of the tube, where it is accessible to most anthophorine bee species. Such long tubes function partly to raise the flower above the rosette of basal leaves, as well as being reservoirs of nectar.

Measurable volumes of nectar are produced in most species, and volume correlates with the length of the

hollow part of the tube (Tables 2, 4). The long-tubed *Babiana ringens* and *B. thunbergii* produced the most nectar, 25.0–28.0 µl in the former in flowers undisturbed by pollinators (the perianth had not fully expanded when nectar was measured) and 24.0–30.0 µl of nectar in the latter, while flowers of the *B. pygmaea group*, pollinated largely or exclusively by hopliine beetles, have the smallest amounts of measurable fluid or evidently none at all. *Babiana melanops* and *B. rubrocynaea*, with up to 1.8 µl or 1.4 µl of nectar, respectively, are exceptional here, but they have a mixed system, using both hopliines and bees. Species pollinated by large-bodied bees offer nectar mainly in the 0.4–3.6-µl range, but as much as 11.0 µl of nectar was produced by a sample of *B. sambucina*. Most species pollinated by long-proboscid flies and moths offer 1.6–9.6 µl of nectar, considerably less than the 19–30.0 µl of nectar offered by sunbird-pollinated species. Putatively moth-pollinated *B. virginea*, however, is exceptional in offering nectar in the 17.0–22.0-µl range.

Nectar sugar analyses of *Babiana* species indicate that all species offer nectars that are sucrose dominant (sensu Baker & Baker, 1983), with the ratio of sucrose to hexose sugars greater than 1 (Table 4). There is, however, a fair degree of variation in the sucrose to hexose ratio, with the bird-pollinated *B. ringens* and *B. thunbergii* producing nectar with sucrose to hexose ratios between 1.65 and 2.45 (Table 4), whereas some long-proboscid fly–pollinated and bee-pollinated species have ratios above this range, sometimes with the ratio greater than 8 in one sample of *B. sambucina* subsp. *sambucina*, as well as in the putatively moth-pollinated *B. patersoniae*.

As noted in other genera of Iridaceae, including *Gladiolus*, *Hesperantha* Ker Gawl., *Ixia* L., and *Sparaxis*, nectar sugar concentration correlates only to a limited extent with pollinator type (Goldblatt et al., 1998a, 2000a, b, 2001, 2004b; Goldblatt & Manning, 1999, 2000). Thus, species pollinated by nectar-foraging anthophorine bees and *Apis mellifera* have nectar concentrations ranging from 22.0%–42.3% sucrose equivalents with an exceptional more than 50% in *B. tritonioides*. Among these species, *B. fimbriata* (Klatt) Baker and *B. patula* N. E. Br. also stand out in having unusually concentrated nectar for a bee-pollinated species, 40.1%–42.3% sucrose equivalents.

In long-proboscid fly–pollinated species of *Babiana*, nectar concentration is typically in the 24.0%–30.0% range, with a high reading in *B. spathacea* of 32.3% sucrose equivalents. Bird-pollinated species have slightly more dilute nectar, 21.0–25.5 sucrose equivalents, a characteristic noted for this pollination system in other genera of the Iridaceae, including some species of bird-pollinated *Gladiolus* (Goldblatt et al., 1999, 2001).

Moth-pollinated (or presumed moth-pollinated) species produce nectar of 26.0%–31.7% mean sucrose equivalents, lower than that encountered in moth-pollinated species of *Gladiolus* and *Hesperantha*, which, in the latter, may exceed 50% (Goldblatt & Manning, 2002; Goldblatt et al., 2004b).

COMPATIBILITY RELATIONS

Only one species studied, *Babiana tubulosa*, showed full self-compatibility and autogamy. Plants set full complements of mature capsules, evidently containing viable seeds when isolated from visiting insects and without hand pollination. Capsules appeared normal, as did seeds, but we did not conduct viability tests. The remaining seven species for which compatibility relations were examined proved self-incompatible. These included *B. ambigua*, *B. attenuata*, *B. curviscapa*, *B. fourcadei* G. J. Lewis, *B. latifolia* L. Bolus, *B. odorata*, and *B. patula*. No capsules were produced in hand-pollinated flowers. Compatibility relations are known in one more species, the sunbird-pollinated *B. ringens* (Anderson et al., 2005), which is a facultative self-pollinator.

From this limited sampling, we cautiously suggest that self-incompatibility may be the rule in the genus and that facultative autogamy occurs in at least two species with specialized pollination systems, although not in the above two long-proboscid fly–pollinated species, *Babiana curviscapa* and *B. latifolia*.

Discussion

POLLINATOR DIVERSITY

Early observations on the pollination biology of *Babiana* were limited to less than a handful of species. These included visits to *Babiana plicata* Ker Gawl. (probably what is now *B. fragrans* (Jacq.) Ker Gawl.) by the hopliine beetle *Anisonyx ursus*, reported by Scott Elliot (1891), who also noted visits by *A. ursus* to a species he called *B. spathacea* in a population near Cape Town. From the illustration provided, this is probably *B. ambigua*. In addition, Marloth (1898, 1915) first recorded visits to flowers of *B. ringens* by the sunbird, *Nectarinia violacea*, and followed Scott Elliot (1890) in concluding that the species was pollinated by these birds. Until then, this assumption had been based solely on inferences drawn from floral morphology.

These preliminary observations did not even begin to show the diversity of pollination systems in the genus. Vogel (1954), in his mammoth review of pollination systems in southern Africa, concluded that

species of *Babiana* were pollinated predominantly by bees (though he made no personal observations of insect visitors to the genus). He also inferred that *B. tubiflora* was pollinated by the same long-proboscid fly as *Lapeirousia fabricii* (D. Delaroche) Ker Gawl. (i.e., *Moegistorhynchus longirostris*), and that *B. bainesii* probably had this same pollination system, which he called psychophily (pollination by day-flying butterflies), a category in which he included long-proboscid fly pollination. Vogel also recognized that *B. ringens* was pollinated by sunbirds. More recently, *B. thunbergii* (as *Antholyza plicata* L. f.) as well as *B. ringens* were regarded by Rebelo (1987) as pollinated by sunbirds in his review of that pollination system in the Cape flora (i.e., the greater part of the winter-rainfall climate zone flora of southern Africa).

Pollination in *Babiana* is, in fact, considerably more diverse than hitherto suggested. As we have shown for other African genera of the Iridaceae, pollination systems in *Babiana* are closely correlated with floral structure, pigmentation patterns, timing of anthesis, scent characteristics, and nectar secretion (Goldblatt et al., 1995, 2000a, b, 2001, 2004b). In general, the radiation of pollination systems in *Babiana* closely parallels that found in other larger genera of the Iridaceae in southern Africa, in particular, of subfamily Crocoideae (syn. Ixioideae). Pollination by *Apis* and large-bodied and long-tongued anthophorine bees is the most common system in *Babiana* and most likely the ancestral one. The system exactly parallels that recorded in *Gladiolus* (ca. 167 species in southern Africa), which have a pollination system in which a species is dependent on one or two species of anthophorine bee, or sometimes *Anthophora* and worker honeybees, for its pollination (Goldblatt et al., 1998a). The same system has also been reported for the southern African genera *Hesperantha* (ca. 80 species), *Ixia* (ca. 52 species), and *Sparaxis* (15 species) (Goldblatt et al., 2000a, b, 2004b). In *Babiana*, 18 species have been shown to be pollinated by large-bodied and long-tongued anthophorine bees and *Apis mellifera*. Another 35 species have flowers of this type, thus 53 species (58% of the genus) are inferred to share this pollination system. One of these species, *B. fragrans*, seems as often visited and effectively pollinated by hopliine scarab beetles. The presence of a strong floral scent and ample nectar in *B. fragrans* flowers seems to suggest that bee pollination is the more important system in the species. Hopliine visitors may simply be opportunistic, for the relatively small area offered by the outspread tepals seems poorly suited to the activity of the relatively large *Anisonyx ursus* captured on them.

As in *Gladiolus*, bee pollination comprises two contrasting systems (Goldblatt et al., 1998a; Bernhardt & Goldblatt, 2000) with floral characters and floral dimensions correlating with bee size, proboscis length, and foraging behavior. The dominant one is a passive pollination system, discussed above, where bees foraging for nectar in gullet flowers (in the terminology of Faegri & van der Pijl, 1979) passively accumulate loads of pollen on the dorsal part of the head and thorax, subsequently to be transferred to receptive stigmas of other flowers. A second bee pollination system involves flowers with an actinomorphic perianth, radial symmetry, and prominently displayed anthers where bees actively collect pollen, although nectar is also offered. Again, as in *Gladiolus*, the flowers of *Babiana* species with this second bee pollination system are inferred to be secondarily radially symmetric, and the active pollination system is derived and less frequent than the passive one. Another similarity with *Gladiolus* is that bee-pollinated flowers in *Babiana* are usually strongly fragrant. One species, *B. villosula*, has been confirmed to be pollinated by *Apis mellifera* foraging for pollen, and four more species (Table 2) share this type of flower (6% of the genus). One population of *B. melanops* also has this pollination system, in which *Anthophora diversipes* and the anthophorine, *Xylocopa rufitarsis*, actively visit flowers for nectar and pollen. Elsewhere, this species is pollinated exclusively by hopliine beetles (Table 3), and the flowers lack fragrance and nectar. Two species, *B. rubrocyanea* and *B. stricta*, are visited by a combination of hopliines and bees (Table 3), and both offer small quantities of nectar as well as pollen. We regard these two species and *B. fragrans* (see above) as having a mixed or bimodal pollination system (Table 5).

Like other large genera of subfamily Crocoideae, pollination by long-proboscid flies is important in *Babiana*. Thirteen taxa (12 species and one subspecies) have been recorded to be visited by long-proboscid nemestrinids, species of *Prosoeca* or *Moegistorhynchus longirostris*, all of which can be seen to brush against anthers and stigmas and carry visible loads of pollen (Table 3). This pollination system is inferred for an additional five species with virtually identical floral presentation. Thus, a total of 18 taxa (20% of the genus) are pollinated by long-proboscid flies, most of them exclusively. Of these, only *B. ranzijliae* may have a mixed system, with some populations with a shorter perianth tube being effectively pollinated by bees as well as long-proboscid flies, although the bees were not able to access nectar. Pollination by long-proboscid flies is the second most important pollination strategy in both southern African *Gladiolus* and *Babiana*, with 18% of

Table 5. Frequency and taxonomic distribution of pollination systems in *Babiana*. The genus comprises 90 species distributed in three sections, all assumed to be monophyletic (Goldblatt & Manning, 2007); n/a = not applicable. For scoring purposes, *B. sambucina* subsp. *sambucina* and subsp. *longibracteata* are scored separately with the result that a total of 91 species are included in the table.

Pollination system	Total species confirmed	Total confirmed and inferred	Taxonomic sections	Taxonomic series
Large-bodied, long-tongued bees (Apidae s.l.)	18	53 (58%)	3	8
Short-tongued (or pollen-collecting) bees	1	5 (6%)	2	2
Long-proboscid fly	13	18 (20%)	2	4
Passerine bird	2	3 (3%)	2	2
Moth	1	3 (3%)	2	4
Hopliine beetle	6	6 (7%)	2	3
Hopliine beetle/pollen-collecting female bee	3	3 (3%)	1	1
Total	44	91 (100%)	n/a	n/a

Gladiolus species in the subcontinent having this pollination system (Goldblatt et al., 2001). Long-proboscid fly pollination also occurs in several other genera of Iridaceae subfamily Crocoideae, including *Geissorhiza*, *Hesperantha*, *Lapeirousia*, *Radinosiphon* N. E. Br., *Romulea* Maratt., *Tritonia* Ker Gawl., *Tritoniopsis* L. Bolus (Goldblatt et al., 1995; Manning & Goldblatt, 1996, 2005; Goldblatt & Manning, 1999, 2000), and *Watsonia* Mill. (Manning et al., 1999; Bernhardt & Goldblatt, 2006).

Pollination by hopliine beetles in *Babiana melanops*, *B. pygmaea*, *B. regia*, *B. rubrocyanea*, *B. stricta*, and *B. villosa* exploits the same set of floral characters described by Goldblatt et al. in southern Africa (1998b, 2000a, b) for *Aristea* Sol. in Aiton, *Ixia*, and *Sparaxis* and by Goldblatt and Bernhardt (1999) for *Moraea* Mill. The flowers of such species are exceptional in *Babiana* in being actinomorphic, usually having a comparatively short style, often darkly colored filaments, and anthers or pollen either brownish or violet to almost black; in *B. melanops*, *B. stricta*, and *B. villosa*, the flowers also have an expanded, dark-colored connective. The dark stamens or parts of stamens may contribute to the contrasting dark pigmentation common in beetle-pollinated species (Steiner, 1998; Goldblatt et al., 1998b, 2000b). Alternatively, unusually colored pollen may be a form of camouflage, as suggested by Goldblatt and Manning (1999), in which unconventionally colored pollen may not appear to be an edible resource for foraging female bees.

The situation in *Babiana melanops* is puzzling. One population at the southern extremity of its range has radially symmetric flowers with a floral odor and nectar and is visited actively by bee species, as well as hopliine scarabs (Table 3). Other populations have odorless flowers that lack nectar and are visited exclusively by hopliines. An argument might be made for infraspecific status or even a separate species for the population, but other features of the population accord so closely with nectarless and odorless populations growing a short distance to the north that these alternatives have not been taken seriously. In some other predominantly hopliine-pollinated species, short-tongued bees or tabanid flies with probosces 4–5 mm long may occasionally be found visiting their flowers. Because hopliine flowers are salver-shaped or shallow bowl-shaped (sensu Faegri & van der Pijl, 1979), this is not surprising as the bright colors of the spreading tepals are readily seen and readily investigated by passing insects other than hopliines.

Pollination by night-flying moths and sunbirds is relatively unimportant in *Babiana*, and we infer that just six species have flowers adapted for these pollination systems, which appear to have evolved convergently, three times for moth pollination and twice for sunbird pollination (Table 5).

In summary, there are six discrete pollination systems in *Babiana*, a genus of only 90 species. Most of the species have a system using just one class of pollinator and, in the case of long-proboscid flies, usually just a single pollinator species. Species using two classes of pollinator are few and may either represent a transition from one system to another or have stabilized to exploit two different specialized systems. *Babiana stricta* is the most prominent example of a species with a mixed system; the spreading tepals of the radially symmetric perianth are often seen with resident hopliine beetles, while *Apis* and halictid bees are frequently observed foraging on the lightly scented flowers that offer

nectar as well as pollen rewards. The transition seems complete in the subspecies of *B. sambucina*, in which subsp. *sambucina* is consistently pollinated by bees and no population shows evidence of the shift to long-proboscid fly pollination characteristic of subsp. *longibracteata*. In *B. melanops*, different populations with small floral differences, whether scented and with nectar or not, permit different pollinator profiles, perhaps an example of a pollinator shift taking place today. The dynamics of these mixed systems are unknown, and shifts toward one or the other may track shifts in the prevalence of one or the other of the two groups of pollinators in response to changing environmental conditions.

FLORAL SYNDROMES AND SPECIALIST POLLINATION

In the debate concerning the importance of specialized versus generalist pollination and the utility of the floral syndrome concept (Waser, 1996), *Babiana* provides numerous examples of specialized flowers exhibiting discrete sets of floral traits (pollination syndromes). Hours of observation on various species with each set of traits repeatedly show the same result: a close link between floral presentation and pollinator class. In this genus, visits by what might be termed inappropriate visitors are rare, and few of them actually accomplish effective pollen transfer. The example of the long-proboscid *Prosoeca peringueyi* taking pollen from flowers of (apparently) large bee-pollinated *B. cedarbergensis* illustrates this dramatically. Several flies observed and captured while taking nectar failed to contact anthers and stigmas, and examination of the bodies of captured flies failed to show the presence of pollen of the species. *Babiana* provides one more example of a genus in a plant family in which pollination syndromes are highly predictive of the most effective (or only) pollinator and thus supports Johnson and Steiner's (2000) defense of the syndrome concept and the prevalence of specialist pollinator systems, at least in the tropics and the southern African temperate zone.

The fact that so many *Babiana* species depend on just one pollinator species is especially striking. Some 10 taxa are pollinated exclusively by *Prosoeca peringueyi* and three more only by *Prosoeca* sp. 1. Another four species are evidently pollinated only by *Moegistorhynchus longirostris*. These flies are clearly keystone species in the ecology of western southern Africa (Goldblatt et al., 1995; Goldblatt & Manning, 2000). Their extinction locally means one of two things: either failure to reproduce by seed (if self-incompatible or requiring mechanical pollen transfer even if self-compatible), or gradual inbreeding depression for self-compatible and autogamous species. Not surprisingly, several such specialist species sometimes have fail-safe mechanisms and are facultatively autogamous. For the few long-proboscid fly–pollinated species for which compatibility relations are known, several show autogamy. *Lapeirousia jacquinii* and *L. oreogena* are prominent examples of such species in the *P. peringueyi* pollination guild (Goldblatt & Manning, 2000). *Babiana tubulosa* provides an example of facultative autogamy in the *M. longirostris* pollination guild, in which some other members (e.g., *L. anceps*) also exhibit this feature. Another species, the sunbird-pollinated *B. ringens*, is also self-compatible and shows a high self-pollination rate when access to pollinators is prevented (Anderson et al., 2005). We anticipate that further study of compatibility relations in species with few or single species pollinators will expand the list of facultative autogams in *Babiana*.

NECTAR VARIATION

Nectar chemical composition of *Babiana* species shows none of the variation in nectar sugar proportions sometimes correlated with differences in pollinators (e.g., Baker & Baker, 1983, 1990). Nectars are uniformly sucrose-dominant, and variation is largely in volume, with larger flowers with large-bodied pollinators producing greater amounts of nectar. Thus, sunbird-pollinated species produce the largest volumes of nectar, usually followed by long-proboscid fly–pollinated and moth-pollinated species. Beetle flowers produce little or no measurable nectar. Nectar concentration shows the same patterns observed in other genera of the Iridaceae (Bernhard & Goldblatt, 2006). Bee- and settling moth-pollinated flowers produce the most concentrated nectar, followed by long-proboscid fly–pollinated flowers. Bird-pollinated flowers often produce relatively dilute nectar (Goldblatt et al., 1999), and *Babiana* species in this category also show this trend. *Babiana* species pollinated by sunbirds do not show any evidence of the predicted trend toward the production of hexose-rich nectar found, for example, in a few species of bird-pollinated Iridaceae in the genera *Gladiolus*, *Klattia* Baker, *Witsenia* Thunb. (Goldblatt et al., 1999, 2001), *Chasmanthe* N. E. Br. (Goldblatt et al., 2004a), and *Tritoniopsis* (Manning & Goldblatt, 2005). Nectar sugar characteristics thus appear to be the result of phylogenetic history and are not pollinator driven, a conclusion consistent with patterns demonstrated, for example, by van Wyk et al. (1993) and Barnes et al. (1995) in some groups of Asphodelaceae and Ericaceae.

FREQUENCY OF POLLINATION SHIFTS

Passive pollen transfer by large-bodied bees appears ancestral in the genus, based on outgroup comparison (Davies et al., 2004; Goldblatt et al., 2006) and on its occurrence in the putatively least specialized species in the genus (Goldblatt & Manning, 2007). It is also inferred to be the primary pollination mechanism in some 58% of *Babiana* species, but we hypothesize that shifts to other strategies have occurred multiple times each (Table 5). Comparing the pollination system to our morphology-based classification of the genus (Goldblatt & Manning, 2007), we conclude that long-proboscid fly pollination, present in 20% of the species, arose independently four times. For exclusive hopliine beetle pollination, which is present in 7% of the genus, we infer a minumum of three shifts to this system. For pollination by pollen-collecting female bees, present in 6% of the genus, we infer two shifts, while moth pollination, present in three species, arose independently in each, and sunbird pollination, present in three species, arose twice. Therefore, because of the taxonomic distribution of derived pollination systems across the genus, we postulate that there have been multiple shifts of each of them and a minimum of 14 shifts in the genus, thus an average of one shift for every six species.

The diversity of pollination systems in *Babiana* mirrors that in other large genera of Iridaceae subfamily Crocoideae and is most comparable to the situation in *Gladiolus*. In that genus, there are 165 species in southern Africa and just one more pollination system (Goldblatt et al., 2001). Some 27 shifts in pollination system have been postulated for the genus in southern Africa, an average of one shift for every five species. Proportions of the different pollination systems differ marginally: long-proboscid fly pollination (18%); sunbird pollination (12%); moth pollination (7%); large butterfly pollination, which is absent in *Babiana* (5%); pollen-collecting bee pollination (2%); and beetle pollination (< 1%) of species. Clearly, hopliine beetle pollination has been exploited to a greater extent in *Babiana*, and both sunbird and moth pollination are proportionately less exploited. The proportions of long-proboscid fly pollination in the two genera are markedly similar, 18% in *Gladiolus* and 20% in *Babiana*.

PATTERNS OF STRUCTURAL CHANGE IN FLORAL MORPHOLOGY

A change in pollinator from an insect with relatively short mouthparts, such as apid bees, short-proboscid flies, and possibly other polyphagic taxa, to one with longer mouthparts, such as long-proboscid nemestrinids or sunbirds, may seem relatively insignificant. After all, this at first appears to be a moderate shift and one only of degree. It should, however, be remembered that a shift to a long-proboscid pollinator means a change from a system where several species of bee and fly, sometimes associated with hopliine beetles, may pollinate a species to one where only a single organism is the primary pollinator. Long-proboscid flies are particularly effective pollinators because there are relatively few species that offer them a reliable source of nectar and they usually carry heavy loads of pollen at specific parts of their bodies, limiting or excluding stigma clogging. The shorter-tongued insects, however, visit many species, sometimes in a non-constant pattern, and they usually carry mixed loads of pollen that are often randomly scattered over their bodies. These contrasting patterns of pollination must have important consequences for the reproductive systems of the two groups. One must conclude that a shift from a generalist to a specialist pollination system does have important consequences for the radiation of a genus, although the shift in morphological and physiological characters seems modest at best. Furthermore, *Babiana* remains the most compelling example yet among the genera of Iridaceae of an instance in which a distinct specialized pollination system, the *Prosoeca peringueyi* system, might have been stimulated to develop (Manning & Goldblatt, 1996).

The evolution of hopliine beetle pollination in *Babiana* involves perhaps the most complex set of morphological changes of any pollination shift. These include suppression of nectar production and associated reduction in the diameter of the perianth tube, loss of floral odor, change of perianth from zygomorphic with bilateral symmetry to actinomorphic with radial symmetry, the frequent development of bizarre color contrasts on the tepals, and, in some species of the genus, exaggerated size of the anthers, often combined with unconventional anther and pollen pigmentation. As in *Sparaxis* and *Tritonia*, genera of Iridaceae–Crocoideae that also have species with bilabiate zygomorphic flowers, as well as hopliine-pollinated species with radially symmetric perianths, these apparently unspecialized flowers are believed to be derived from ancestors with bilabiate flowers, and the change is believed to be directly associated with a shift to hopliine pollination (Goldblatt et al., 2000a). This flies against the conventional wisdom of floral evolution from a radial symmetry to zygomorphy with differentiation in size, shape, and orientation of the tepals. These observations suggest that a more dynamic view of floral evolution is required, where gross structural changes should be viewed against the adaptive background with which they are associated, and that outgroup comparison rather than degrees of

developmental complexity more reliably indicate the direction of change.

Lastly, we draw attention to comparisons of floral form and presentation with particular sets of pollinators in other genera of the Iridaceae, especially members of subfamily Crocoideae, where floral form is repeated again and again in different genera. As in *Gladiolus*, short-tubed, bilaterally symmetric gullet flowers, often of predominantly blue or sometimes pink color with pale nectar guides on the lower tepals and with strong floral odor, are pollinated by anthophorine bees and worker honeybees. Similarly colored flowers showing radial symmetry, scented or not, are pollinated by female bees foraging for pollen. Likewise, in *Lapeirousia*, long-tubed gullet or salver-shaped flowers with dark blue or purple pigmentation and pale nectar guides on the lower tepals, or with cream to pink flowers with reddish markings on the lower tepals, are pollinated by the same species of long-proboscid nemestrinid flies as we have expanded documention in *Babiana*. In the same vein, pale-colored, scented flowers that are open at night show the same sets of moth pollinators as do species of *Hesperantha* and *Lapeirousia* with similar flowers. Red flowers pollinated by birds in *Babiana* show the same avian pollinators as do species of *Gladiolus* with similarly pigmented flowers with elongate perianth tubes that are wide in the upper half. These repeated trends in different genera allow us to predict with confidence the pollinators of species in other genera, the floral biology of which is incompletely known.

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A REVISION OF *BOURRERIA* (BORAGINALES, EHRETIACEAE) IN SOUTH AMERICA[1]

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ABSTRACT

A review of South American herbarium specimens of *Bourreria* P. Browne (Boraginales, Ehretiaceae) indicates that five species are readily distinguished. *Bourreria bolivarensis* Gottschling & J. S. Mill. is described as new; *B. baccata* Raf., *B. exsucca* Jacq., and *B. mollis* Standl. have their nomenclature clarified; *B. exsucca* is epitypified; and a neotype has been designated for *Rhamnus cumanensis* Loefl. (= *B. exsucca*). *Bourreria bolivarensis* and *B. exsucca* are common species in the northern South American flora. *Bourreria costaricensis* (Standl.) A. H. Gentry and *B. mollis* are Central American species that have not been previously reported for South America. *Bourreria baccata* is a Caribbean species with the southern end of its distribution on Tobago, but it also occurs in a small, isolated population in the Netherlands Antilles and on the adjacent Venezuelan coast, perhaps resulting from a single long-distance dispersal event. *Bourreria exsucca* and the new species *B. bolivarensis* have their distribution restricted to South America and exhibit schizocarpous fruits. The other three species have drupaceous fruits. All five species have extent of occurrence above the threshold (> 2000 km^2) to be considered vulnerable based on the IUCN Red List categories, and none of the five species that occur in South America appear to be threatened when evaluated solely on their distributions.

Key words: Boraginaceae, *Bourreria*, Colombia, dispersal, distribution, IUCN Red List, schizocarp, taxonomy, Venezuela.

Bourreria P. Browne (Boraginales, Ehretiaceae) comprises approximately 30 species of tropical trees and shrubs that grow mostly in dry scrubby habitats and deciduous forests. A few species such as *B. costaricensis* (Standl.) A. H. Gentry have secondarily colonized predominantly wet habitats. *Bourreria* has its center of diversity in the Caribbean and Central America (Al-Shehbaz, 1991; Miller & Sirot, 1997; Miller, 1999) but also occurs in northwestern Mexico, Florida, and northern South America.

The precise systematic position of *Bourreria* remains unclear at present, since there is still a need for comprehensive phylogenetic analysis of Ehretiaceae. Monophyly of New World *Bourreria* s. str. has been supported by ITS-1 sequence data (Gottschling & Hilger, 2001; Gottschling et al., 2004). The closest relative is *Hilsenbergia* Tausch ex Meisn. from East Africa and Madagascar (Miller, 2003). This is inferred from molecular trees and synapomorphies such as the valvate calyx, the overlapping lamellae on the abaxial surface of the pyrenes (also called endocarpids), and the additional placental ("sterile") chamber found in each pyrene (Gottschling, 2001, 2004; Gottschling & Hilger, 2001).

Basic taxonomy is challenging in *Bourreria* due to the high morphological plasticity of the species, and a comprehensive classification is still lacking. Several species appear closely related, including those with large flowers and corollas to 4 cm in diameter (approximately seven species), while the remaining species have smaller flowers and corollas about 15 mm in diameter or less. However, this grouping may be artificial and has no support from any rigorous phylogenetic analysis (Miller, 1999). Two fruit types are found in *Bourreria*, indehiscent drupes (ca. 25 species) and dry schizocarps (five species). This fruit character is unique in the entire Boraginales and argues unequivocally for the monophyly of the schizocarpous species group. Miers (1869) recognized its distinctiveness and segregated those species as *Crematomia* Miers.

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Table 1. Risk of extinction based on the IUCN Red List categories.

	Total no. of specimens	Total area of occurrence (km^2)	Total extent of occupancy (km^2)
Bourreria baccata	499	2988	2,476,206
B. bolivarensis	20	162	36,089
B. costaricensis	88	540	476,655
B. exsucca	88	666	308,352
B. mollis	180	1188	716,523

A review of herbarium specimens for the preparation of a comprehensive revision of *Bourreria* led to the present re-evaluation of the South American species. *Bourreria exsucca* Jacq. is epitypified, and a neotype has been designated to *Rhamnus cumanensis* Loefl. Furthermore, new records of three otherwise Central American or Caribbean species are reported for the first time from South America, and the new species *B. bolivarensis* Gottschling & J. S. Mill. is described. Hypotheses on intraspecific variability of different organs and the distributions of the plants are presented.

The number of species in South America is relatively low but spans much of the morphological diversity found in *Bourreria*. In this revision, we discuss that the five South American representatives of *Bourreria* are a heterogeneous group of only distantly related species. Thus, the continent appears to have colonized several times independently from Central America and the West Indies, either by exceptional long-distance dispersal events due to their endozoochorous (drupes) or presumptively anemochorous fruits (schizocarps), or simply by migration.

IUCN Conservation Status

Specimen data for all five species of *Bourreria* that occur in South America were reviewed and used to calculate area of occurrence (AOO) and extent of occupancy (EOO) following IUCN (2001), and these values were used to estimate risk of extinction based on the IUCN Red List categories (Table 1). Only two of the five species that occur in South America are endemic, and the other three have significant parts of their ranges in Central America or the West Indies. All five species have EOOs above the threshold (> 2000 km^2) to be considered vulnerable.

Although *Bourreria bolivarensis* has an AOO of 162 (below the < 500 km^2 threshold for endangered), *B. costaricensis*, *B. exsucca*, and *B. mollis* Standl. have AOOs below the 2000 km^2 threshold for vulnerability. This is likely due to a paucity of collections, rather than the rarity in the areas in which they occur. *Bourreria mollis*, for example, has been collected only infrequently south of Honduras, but it is abundant and frequently encountered in Belize and the Yucatán Peninsula of Mexico.

While the dry forest habitats in which four of the five South American species grow face tremendous threat, none of these species seem to have reached a threshold where they can be considered immediately threatened. Surveys of populations in the field will be required to confirm the provisional IUCN Red List assignments presented here (IUCN, 2001).

Key to *Bourreria* Species in South America Based on Floral Characters

1a. Flowers large; corolla mostly > 20 mm; tube long, usually > 15 mm 2
1b. Flowers small; corolla mostly < 20 mm; tube short, < 10 mm 3
2a. Style exserted, > 20 mm; calyx < 10 mm . . . . . 4. *Bourreria exsucca*
2b. Style included, < 15 mm; calyx > 10 mm . . . . . 3. *Bourreria costaricensis*
3a. Filaments at least slightly sericeous at their corolla tube insertion . . . . . 2. *Bourreria bolivarensis*
3b. Filaments glabrous at their corolla tube insertion 4
4a. Style undivided; leaf apex predominantly rounded or obtuse . . . . . 1. *Bourreria baccata*
4b. Style divided; leaf apex predominantly acuminate . . . . . 5. *Bourreria mollis*

Key to *Bourreria* Species in South America Based on Fruit Characters

1a. Fruits dry, schizocarpous (breaking into 4 units at maturity, each mericarpid attached to the gynobase by an apical fiber) 2
1b. Fruits drupaceous 3
2a. Fruits rather compact, with indistinct beak; coastal plants of Colombia and Venezuela . . . . . 4. *Bourreria exsucca*
2b. Fruits rather slender, with prominent beak; Venezuelan plants of forest habitats south of the Orinoco River . . . . . 2. *Bourreria bolivarensis*

3a. Fruits large, > 20 mm . . . . . 3. *Bourreria costaricensis*
3b. Fruits small, < 15 mm 4
4a. Style undivided; leaf apex predominantly rounded or obtuse . . . . . 1. *Bourreria baccata*
4b. Style divided; leaf apex predominantly acuminate . . . . . 5. *Bourreria mollis*

Taxonomy

Bourreria P. Browne, Civ. Nat. Hist. Jamaica: 168. 1756, nom. conserv. TYPE: *Bourreria baccata* Raf.

Morelosia Lex. in La Llave & Lex., Nov. Veg. Descr. 1: 1. 1824. TYPE: *Morelosia huanita* Lex. [= *Bourreria huanita* (Lex.) Hemsl.].

Crematomia Miers, Ann. Mag. Nat. Hist., Ser. IV, 3: 302. 1869. TYPE: *Crematomia cumanensis* (Loefl.) Miers (≡ *Bourreria exsucca* Jacq.).

Trees or shrubs; bark smooth or slightly striate when young, light to dark gray or brown, longitudinally fissured; indument absent or puberulent to velutinous or strigose, trichomes simple, unicellular, eglandular, or rarely glandular. Leaves alternate or fasciculate, deciduous or evergreen, simple, sometimes microphyllous, blade mostly elliptic to obovate, margin entire, texture membranaceous to coriaceous, venation brochidodromous, upper blade surface frequently with multicellular incrustrations; stipules rare; petioles smooth or canaliculate on adaxial surface. Inflorescences terminal, rarely axillary, thyrsoid, fertile branching sympodial, lax or compressed, bracts and bracteoles frequent, leafy or inconspicuous. Flowers actinomorphic, bisexual, often fragrant, sessile or pedicellate; perianth 5-merous, buds ellipsoid or obovoid; calyx synsepalous, persistent, campanulate or tubular, coriaceous or membranaceous, dehiscence regular or irregular, perianth lobes valvate; corolla sympetalous, mostly white, predominantly membranaceous, rotate to funnel-shaped, dehiscence imbricate (quincuncial), tube short or elongate, lobes spreading or reflexed, mostly rounded, alternating with the sepals; stamens 5, antesepalous, epipetalous, well exserted or included, filaments adnate to corolla tube, anthers tetrasporangiate, narrowly elliptic or oblanceolate, dorsifixed, introrse, opening via longitudinal slits; ovary superior, sessile, bicarpellate, syncarpous, conical, with 4 ovules, style terminal, divided or undivided, stigmas 2 or rarely 1, ovary disc round, usually extensive, ovules anatropous, unitegmic. Fruits 4-seeded, indehiscent and drupaceous or schizocarpous and dry, exocarp thin or rarely extensive, mesocarp succulent or dry, endocarp separating into 4 pyrenes (endocarpids), each usually with conspicuous abaxial lamellae, enclosing one seed and an additional sterile chamber, attached by an apical fiber (carpophore, originating from ventral vascular traces) to gynobase (columella) in schizocarpous fruits; testa with transfer cells (Diane et al., 2002); embryo curved, endosperm copious, cotyledons flat. Chromosome number $n = 19$ (Al-Shehbaz, 1991).

1. Bourreria baccata Raf., Sylva Tellur.: 42. 1838. *Cordia bourreria* L., Syst. Nat. ed. 10. 2: 936. 1759. *Ehretia bourreria* (L.) L., Sp. Pl. ed. 2. 1: *Morelosia beurera* Kuntze, Revis. Gen. Pl. 2: 439. 1891. *Bourreria bourreria* (L.) Huth, Helios 11: 133. 1893. TYPE: Jamaica. s. loc., s.d. (fl), *P. Browne s.n.* (lectotype, designated by Stearn, 1971: LINN 254.2!).

[Synonymy includes only names that have been considered for South American populations.]

Bourreria succulenta Jacq., Enum. Syst. Pl.: 14. 1760, nom. inval., Select. Stirp. Amer. Hist.: 44. 1763, descr. Syn. nov. TYPE: tab. 26 in Pt. 2, Observ. Bot., 1767, *N. J. Jacquin* (type, tab. 26 in Jacquin, 1767).

Bourreria recurva Miers, Ann. Mag. Nat. Hist., Ser. IV, 3. 203. 1869. Syn. nov. TYPE: Dominica. Saint John, Prince Rupert's Bay [at Portsmouth], 1792 (fl), *Lane s.n.* (holotype, BM 85833!; isotype, BM 85830!).

Trees or rarely shrubs, to 6 m, glabrous or with few scattered trichomes, lenticels few or absent. Leaves alternate, blade (0.6–)1.2–10.0(–13.4) × (0.4–)0.9–5.0(–7.7) cm, elliptic to obovate, frequently asymmetric, rarely widely obovate or ovate, texture coriaceous to membranaceous, primary vein sometimes very distinctively prominent, but also rather smooth, secondary veins (3)4 to 7(9), the tertiary veins reticulate or sometimes percurrent, occasionally inconspicuous, adaxial surface glabrous or with few trichomes at veins, frequently with multicelluar incrustrations, abaxial surface glabrous or with few trichomes at veins, apex rounded, acute, or apiculate, occasionally obtuse, retuse, or emarginate, base acute or cuneate, frequently asymmetric, sometimes decurrent, rarely obtuse; stipules absent; petioles (0.1–)0.2–1.1(–1.5) cm, slender, rarely robust, canaliculate on adaxial surface, rarely rather smooth, glabrous or with few scattered trichomes. Inflorescences 4.5–12(–17.5) cm, terminal or rarely axillary, comprising usually to 10 flowers, but occasionally more (to more than 50), fertile branching sympodial, branches slender to robust, glabrous or with few trichomes, bracts frequent, leafy, bracteoles rare, if present leafy or small, lanceolate, and velutinous. Flowers fragrant, pedicels to 11 mm, but mostly shorter, buds widely obovoid, slightly apiculate; calyx (5–)6–8 mm, campanulate or sometimes tubular, coriaceous, indurate and frequently striate in fruit, glabrous or with few

trichomes outside, tomentose at tips inside, sometimes only at margin, usually regularly divided, but irregularly (into 3 + 2 or occasionally 2 + 2 + 1) almost to base in fruit, lobes 3 to 5, 1–3 mm, acute; corolla 9–13(–14) mm, funnel-shaped, membranaceous, white, the tube 5–8(–9) mm, funnel-shaped or rarely tubular, sometimes broadened at base, glabrous on both surfaces, the lobes 3–6 mm, rounded or obtuse, glabrous, papillose, slightly strigose outside, glabrous inside; stamens well or rarely slightly exserted, the filaments 8–12(–13) mm, glabrous, adnate to corolla tube for 3–6(–8) mm, anthers 1–2 mm; ovary glabrous, style (6–)7–10 mm, well exserted, undivided or rarely branches to 1 mm long, stigma capitate, sometimes significantly broader than width of style, ovary disc present. Fruits 6–10 × (7–)9–11 × 5–8 mm, drupaceous, widely to depressed ovoid, red, orange, or yellow, exocarp thin, mesocarp succulent, voluminous, pyrenes 4–6 × 2–4 × 2–3 mm, shape 1/4 of a sphere, abaxial surface with 5 to 6 conspicuous lamellae and additionally with some vertical ridges.

Distribution and habitat. *Bourreria baccata* is a widely distributed Caribbean species ranging from Tobago through the Lesser Antilles to the Greater Antilles and Mexico. An isolated population can be found on the Netherlands Antilles, specifically in Curaçao, Bonaire, and Aruba, with a few records from the adjacent Venezuelan mainland as well. *Bourreria baccata* occurs from sea level to elevations of 300 m.

IUCN Red List category. This species is known on the South American mainland from only two Venezuelan collections, but it occurs on the Netherlands Antilles and Trinidad and Tobago, and it is widespread and commonly collected throughout the West Indies. It is provisionally assigned an IUCN status of LC (Least Concern) (IUCN, 2001).

Notes. The description given here refers to material from the Netherlands Antilles and Venezuela, where the plants have smaller leaves generally, fewer flowers in the inflorescence, and larger flowers than found in the Lesser Antilles. *Bourreria baccata* is highly variable (Stearn, 1971) in leaf shape and indument, but especially in the sizes of the different organs. It remains to be determined whether *B. baccata* is really distinct from other Caribbean species such as *B. virgata* (Swartz) G. Don. Schulz (1911) used the depth of the division of the style to differentiate between the Caribbean species. However, this character can be variable within species of *Bourreria* (Gottschling & Miller, 2007), with undivided styles seen in most individuals of *B. baccata*, but occasionally individuals with divided styles occur (e.g., *Arnoldo 1193*, GH), which are rarely on the same plant (*von Proosdij et al. 519*, GH).

Additional specimens examined. NETHERLANDS ANTILLES. **Aruba:** Spanish Lagoon, *Chamberlaine 36* (GH). **Bonaire:** Ceru Largu, *Arnoldo-Broeders 3730* (A); Montagne, *Stoffers 873* (A). **Curaçao:** *Arnoldo 1193* (A), *Curran & Haman 49* (BM, CAL, F, GH, K, S), *Curran & Haman 243* (GH), *Killip & Smith 21057* (BM, F, GH), *Stoffers 216* (A, GH); Soto, *Arnoldo-Broeders 3799* (GH, NY); near Willemstad, *Britton & Shafer 2965* (F, GH); Jan Thiel, *Morton s.n. 1970* (BM); Christoffelpark, *van Proosdij et al. 519* (A); St. Willibrord, *Stoffers 373* (K). TRINIDAD AND TOBAGO. **Tobago:** near Stoer Bay, *Broadway 9166* (BM, GH, MO); Bacolet, *Eggers 5517* (GH, K, S), *Howard 10455* (BM, GH), *Purseglove P.6207* (K, US); Pigeon Point, *Philcox & Raynal 7898* (K). VENEZUELA. **Falcón:** San Rafel, *Bellesteros MB358* (MA); Santa Ana, *Steyermark & Brown 94596* (GH, P); Silva, *Steyermark & Manara 110383* (MO, VEN); Paraguaná, *Tamayo 799* (F).

2. Bourreria bolivarensis Gottschling & J. S. Mill., sp. nov. TYPE: Venezuela. Bolívar: San Pedro de las Dos Bocas, 7 km N of jct. of Río Paragua and Caroni, 7°00′N, 62°54′W, 200 m, 27 July 1978 (fl, fr), *R. Liesner & A. C. Gonzáles 5719* (holotype, MO 2742482!). Figure 1.

Arbor vel frutex usque ad 12 m alta. Lamina glabra, elliptica, 2–8 cm longa, 1–4 cm lata, apice basique acuta; folia petiolo ca. 0.2–0.9 cm longo, glabro. Inflorescentia terminalis, thyrsoidea. Flos fragrans; calyce 4–7 mm longo, intus tomentoso; corolla alba, campanulata, 12–18 mm longa, 5-loba, tubo 3–6 mm longo, lobulis ovatis, 6–10 mm longis; filamentis ad insertionem puberulis. Fructus exsuccus, elongato-pyriformis, 13–20 mm altus.

Trees or rarely shrubs, to 12(–20) m, glabrous or varying tomentose, lenticels rare. Leaves fasciculate or alternate, blade (0.4–)2.0–8.0(–11.5) × (0.3–)1.0–4.0(–6.0) cm, elliptic to obovate, sometimes asymmetric, rarely ovate or widely elliptic to widely obovate, apex obtuse, acute, or apiculate, occasionally acuminate or retuse, base acute or obtuse, occasionally decurrent or asymmetric, texture membranaceous to coriaceous, primary vein prominent or sometimes rather smooth, secondary veins 4 to 6(8), tertiary veins reticulate or sometimes percurrent, adaxial surface glabrous or with few trichomes, rarely with multicelluar incrustrations, abaxial surface glabrous or with few trichomes; stipules absent; petioles 0.2–0.9(–1.4) cm, slender, rarely robust, smooth or adaxially canaliculate, glabrous to varying tomentose. Inflorescences 3–9(–12) cm, terminal or sometimes axillary, comprising 5 to 20(30) flowers, fertile branching sympodial, branches slender or occasionally robust, glabrous to varying tomentose, bracts frequent, leafy, bracteoles rare, if present leafy or small, lanceolate, velutinous. Flowers fragrant, pedicel to 6 mm, but mostly shorter, buds widely obovoid, distinctively apiculate; calyx campanulate, 4–7 mm, coriaceous to

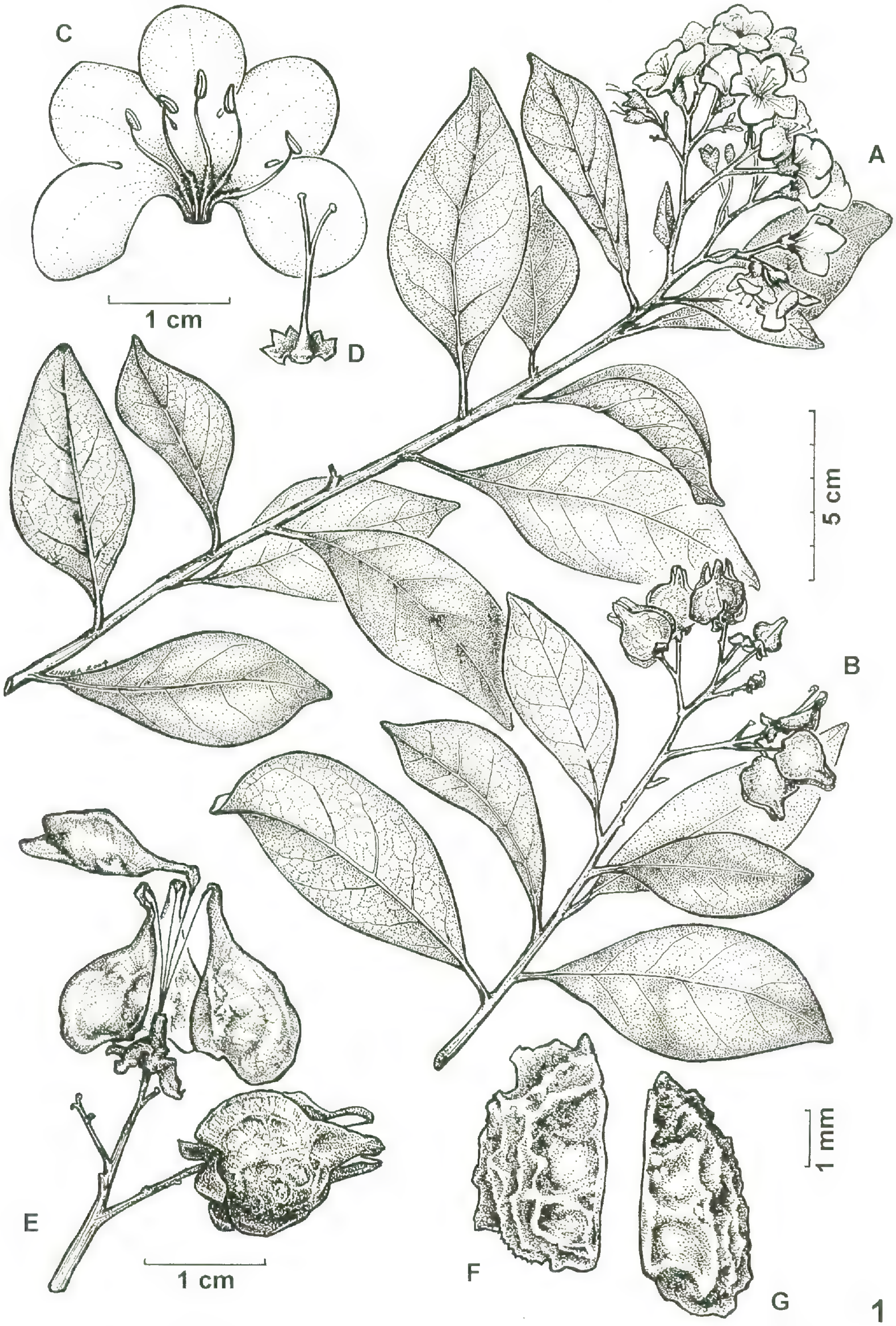

Figure 1. *Bourreria bolivarensis* Gottschling & J. S. Mill. —A. Flowering branch. —B. Fruiting branch. —C. Open corolla with stamens (note the hairy filaments at the point of insertion). —D. Open calyx with gynoecium. —E. Schizocarpous fruits (note the single mericarpids with the apical fiber attached to the gynobase). —F, G. External surface of pyrene showing overlapping lamellae. A, *Davidse 4481* (MO); B and E, *Liesner & Gonzáles 5719* (MO); C and D, *Gentry et al. 10647* (MO); F and G, *Colella & Melina 1413* (MO).

membranaceous, sometimes indurate in fruit, glabrous or with few scattered trichomes outside, with few trichomes to tomentose at tips inside, rather regularly divided (almost to base in fruit), lobes (2)3 to 4, 1–3 mm, acute or acuminate, rarely obtuse, frequently brown and dry at tips, reflexed in fruit; corolla (10–)12–18(–20) mm, campanulate, membranaceous, glabrous on both surfaces, white or creamy, tube 3–6(–8) mm, funnel-shaped to campanulate, lobes (3–)6–10(–12) mm, rounded; stamens well exserted, filaments 9–13(–17) mm, occasionally with some glandular trichomes, sericeous or at least with few trichomes at point of insertion for 1–3 mm, adnate to corolla tube for 2–4 mm, anthers 2–3 mm; ovary glabrous, style (8–)9–14(–17) mm, well exserted, divided, branches 1–4 mm, stigma capitate, ovary disc present. Fruits (11–)13–20 × (10–)12–19 × (8–)10–14 mm, schizocarpous, distinctively 4-angled, elongate pyriform, brown, gynobase 2–3 mm, carpophore (7–)9–12 mm, exocarp thin, mesocarp spongiose, dry, pyrenes 4–6(–7) × 2–3 × 2 mm, shape 1/4 of a sphere, abaxial surface with 3 to 4 inconspicuous lamellae, reticulate.

Distribution and habitat. *Bourreria bolivarensis* grows in tropical wet forests, gallery forests, and adjacent savannahs in elevations 20–700 m and is geographically isolated from *B. exsucca* by the valley of the Orinoco River.

IUCN Red List category. This species is widely distributed in southeastern Venezuela, and its EOO is sufficient to provisionally assign this the IUCN status of LC. Although its AOO of 162 km^2 is below the threshold of 500 km^2, which would indicate EN (Endangered) status, this most likely reflects the paucity of collections made in the area and not the rarity of the plant; additional fieldwork will be necessary to confirm this provisional assignment (IUCN, 2001).

Notes. *Bourreria bolivarensis* may be the sister species of *B. exsucca*. Both are similar in appearance, but the most important difference between them is the distinctively shorter corolla tube of *B. bolivarensis* (3–6 mm vs. 15–19 mm in *B. exsucca*). Furthermore, fruits of *B. bolivarensis* appear more slender and have a more prominent beak than those of *B. exsucca*. The Guayana region south of the Orinoco River differs environmentally from coastal Venezuela and Colombia. The peculiar montane physiography of the Guayana region influences many of its floristic and ecological features (Berry et al., 1995). Such different habitat preferences also separate *B. bolivarensis* and *B. exsucca*.

Paratypes. VENEZUELA. **Bolívar:** betw. Botanamo & Comino, *Bernardi 8055* (K); Sifontes, 3 km NE of Campto, *M. Colella & G. Molina 1413* (MO); 10 km SW of Río Aro, betw. Caicara & Ciudad Bolívar, *G. Davidse 4481* (MO); Caroní, *W. A. Diaz 3001* (MO); Piar, *C. Benítez & W. G. D'Arcy 5303* (MO), *Fernandez 2779* (MO); 10 km E of Ciudad Bolívar, *A. H. Gentry et al. 10411* (MO); Tumeremo to Anacoco (N side of Cuyuni River), 72 km from Guyana frontier at Anacoco, *A. H. Gentry et al. 10647* (MO); 18 km N of Upata, *A. H. Gentry & P. E. Berry 14921* (AAU, MO); Rocio, *B. Stergios et al. 3516* (MO); Carr. Casa Blanca (Tumeremo), *B. Stergios et al. 3824* (MO); Reserva Forestal Imataca, *B. Stergios et al. 3854* (MO), *B. Stergios et al. 3866* (MO); betw. Upata & Altagracia, *J. A. Steyermark 57726* (F); E of Miamo, *J. A. Steyermark 88217* (F); La Paragua, *L. Williams 12803* (F, S, U).

3. Bourreria costaricensis (Standl.) A. H. Gentry, Phytologia 26: 67. 1973. *Schlegelia costaricensis* Standl., Publ. Field Mus. Nat. Hist., Bot. Ser. 18: 1128. 1938. TYPE: Costa Rica. Alajuela: Cataratas de San Ramón, 6 Mar. 1931 (fl), *A. M. Brenes 13570* (holotype, F 857116!; isotype, NY 22213!).

Bourreria panamensis I. M. Johnst., J. Arnold Arbor. 29: 229. 1948. *Bourreria superba* var. *glabra* Schery, Ann. Missouri Bot. Gard. 29: 366. 1942. TYPE: Panama. Bocas del Toro: Laguna de Chiriquí on Isla Colón, 3 June 1941 (fl), *H. v. Wedel 2472* (holotype, GH 94387 not seen; isotypes, MO 1245306!, NY 126858!).

Trees, to 17 m, glabrous, lenticels absent. Leaves alternate, rarely subopposite, blade 7.0–17.5 × 5.1–9.7 cm, elliptic to widely obovate, frequently asymmetric, apex acuminate, but also apiculate, rounded, or retuse, base cuneate or acute, frequently asymmetric, texture coriaceous, rarely membranaceous, primary vein prominent, secondary veins 4 to 5(7), tertiary veins reticulate or rarely percurrent, both surfaces glabrous; stipules absent; petiole 1.0–2.5 cm, robust, canaliculate on adaxial surface, glabrous. Inflorescences 8 cm, terminal or rarely axillary, comprising up to 10 flowers, but frequently only 1 to 3 developed, fertile branching sympodial, branches robust, glabrous, bracts frequent, leafy, bracteoles absent. Flowers fragrant, pedicels to 15 mm; calyx 12–17 mm, campanulate, coriaceous, indurate and striate in fruit, glabrous outside, tomentose at tips inside, irregularly divided (into 3 + 2 lobes) almost to base in fruit or rarely rather regularly divided, lobes 4–6 mm, acute; corolla 38–47 mm, funnel-shaped, membranaceous, white or creamy, papillose outside, tube 20–25 mm, funnel-shaped, glabrous or with some curly trichomes (especially at level of anthers) inside, lobes 17 mm, rounded or obtuse, papillose inside; stamens included, filaments 15 mm, adnate to corolla tube for 4 mm, hirsute at point of insertion, anthers 2–3 mm; ovary glabrous, style 12–14 mm, included, branches 2–3 mm, stigma capitate, often significantly broader than width of style, ovary disc present. Fruits 28 × 33 × 10 mm, drupaceous, globose when young, later cylindrical, exocarp very extensive, mesocarp scanty, pyrenes 20 × 12 × 6 mm, shape of half of

a cylinder, abaxial surface with 5 to 7 conspicuous lamellae, additionally with some vertical ridges.

Distribution and habitat. *Bourreria costaricensis* occurs mainly at elevations between 800 and 1650 m in Nicaragua, Costa Rica, and Panama, with a few records also from Colombia. Its possible occurrence in Ecuador is inferred from one questionable record (*Rubio & Quelal 1444*, MO), which has only one very small flower and is tentatively assigned to this species.

IUCN Red List category. Although known from only a few widely scattered localities in Colombia and possibly Ecuador, this species is frequently collected in several protected areas in Costa Rica. Although its AOO is below the threshold for VU (Vulnerable), the combination of its large EOO with multiple populations that are known from protected areas support a provisional assignment of LC (IUCN, 2001).

Notes. *Bourreria costaricensis* is distinct in its unusually large flowers, and it has the largest drupes of any species of *Bourreria*. The variability of the species in size of vegetative and generative organs appears greater on the Central American mainland than in South America, as can be inferred from the small amount of South American collections. The closest relative is probably *B. grandicalyx* J. S. Mill. & Sirot. and their ancestor might have colonized secondarily wet forest habitats of southern Central America and South America. *Bourreria costaricensis* and *B. grandicalyx* appear to be derived from similar plants as *B. mollis* by enlargement of flowers and fruits. The relationship to other species with large flowers and/or drupes of *Bourreria* such as *B. moaensis* Britton from Cuba and *B. huanita* (Lex.) Hemsl. from Central America remains to be determined. However, the synonymous *B. panamensis* I. M. Johnst. is clearly not closely related to *B. superba* I. M. Johnst. from northern Mexico, even though Woodson and Schery (1942) initially described it as a variety of *B. superba*. The Mexican species has a densely velutinous interior calyx, which it shares with *B. huanita*, but which is not present in *B. costaricensis*.

Additional specimens examined. COLOMBIA. **Chocó:** Bahía Solano, *Espina et al. 2860* (MO); Nuqui-Pangui, *Gómez et al. 389* (HUA, MO). **Valle del Cauca:** Isla de Gorgona, *Cabrera 3241* (CUVC).

4. Bourreria exsucca Jacq., Enum. Syst. Pl.: 14. 1760, nom. inval., Select. Stirp. Amer. Hist.: 45. 1763, descr. *Ehretia exsucca* (Jacq.) L., Sp. Pl. ed. 2. 1: 275. 1762. *Morelosia exsucca* (Jacq.) Kuntze, Revis. Gen. Pl. 2: 439. 1891. TYPE: tab. 173, fig. 17 in Select. Stirp. Amer. Hist., 1763, V. *J. Jacquin* (type, tab. 173, fig. 17 in Jacquin, 1763). [EPITYPE: Colombia. Bolívar: Isla Barú, 10°09′N, 75°41′W, 5 m, 6 Aug. 1985 (fl, fr), *J. L. Zarucchi & H. Cuadros 3951* (epitype, designated here, MO 3506236!; duplicate, JBGP not seen).] Figure 2A.

Rhamnus cumanensis Loefl., Iter Hispan.: 182. 1758. Syn. nov. *Crematomia cumanensis* (Loefl.) Miers, Ann. Mag. Nat. Hist., Ser. IV, 3. 302. 1869. TYPE: Venezuela. Nueva Esparta: Isla de Margarita, El Valle, 15 July 1988 (fl, fr), *J. R. Johnston 86* (neotype, designated here, GH!). Figure 2B.

Crematomia guildingiana Miers, Ann. Mag. Nat. Hist., Ser. IV, 3: 304. 1869. *Morelosia guildingiana* (Miers) Kuntze, Revis. Gen. Pl. 2: 439. 1891. TYPE: Grenadines. Saint Vincent: Botanical Garden (cult., from the South American mainland), *L. Guilding s.n.* (holotype, K!).

Crematomia jacquiniana Miers, Ann. Mag. Nat. Hist., Ser. IV, 3. 305. 1869. Syn. nov. TYPE: Colombia. Bolívar: Cartagena, s.d. (fl), *N. J. Jacquin s.n.* (holotype, BM 85819!).

Crematomia kunthiana Miers, Ann. Mag. Nat. Hist., Ser. IV, 3: 311. 1869. *Bourreria cumanensis* var. *kunthiana* (Miers) O. E. Schulz, in Urb., Symb. Antill. 7: 50. 1911. TYPE: Venezuela. Sucre: near Cumaná. Llanos de Barcellona, 1799 (fl), *A. v. Humboldt & A. Bonpland 101* (holotype, P-Bonpl., IDC 6209 63/19).

Trees or rarely shrubs, to 12 m, indument absent or rarely varying tomentose, lenticels frequent. Leaves alternate or fasciculate, blade (0.5–)0.8–7.3(–11.2) × (0.2–)0.5–4.0(–7.4) cm, elliptic to obovate, ovate, occasionally asymmetric, rarely widely elliptic to widely obovate, apex obtuse, acute, or apiculate, sometimes acuminate, rounded, retuse, or emarginate, base acute or obtuse, occasionally decurrent or asymmetric, texture membranaceous to slightly coriaceous, primary vein prominent or sometimes rather smooth, secondary veins 4 to 7(9), tertiary veins reticulate or sometimes percurrent, occasionally inconspicuous, adaxial surface glabrous or with few scattered trichomes, frequently with multicelluar incrustrations, rarely tomentose or somewhat scabrid, abaxial surface glabrous or with few trichomes at veins, rarely tomentose; stipules absent; petiole (0–)0.1–1.4(–1.8) cm, slender or robust, canaliculate on adaxial surface, glabrous or with few trichomes, rarely tomentose. Inflorescence (3–)6.5–10.0(–13) mm, terminal or sometimes axillary, comprising 15 to 20(30) flowers, fertile branching sympodial, sometimes compressed, branches slender or robust, glabrous or with few scattered trichomes, rarely tomentose, bracts frequent, leafy, bracteoles rare, if present leafy or small, lanceolate, and velutinous. Flowers fragrant, pedicels to 8 mm, but mostly shorter, buds ellipsoid or obovoid, distinctively apiculate; calyx (5–)7–9

Figure 2. —A. Epitype of *Bourreria exsucca* Jacq. (*Zarucchi & Cuadros 3951*, MO 3506236). —B. Neotype of *Bourreria cumanensis* (Loefl.) O. E. Schulz (*Johnston 86*, GH).

(–11) mm, campanulate, coriaceous to membranaceous, indurate and sometimes striate in fruit, glabrous or with few scattered trichomes outside, tomentose at tips inside, rather irregularly divided (almost to the base in fruit), lobes 2 to 4(5), 1–4 mm, acute or acuminate, rarely obtuse, frequently brown and dry at tips, reflexed in fruit; corolla (20–)24–30(–40) mm, funnel-shaped or sometimes campanulate, membranaceous, glabrous on both surfaces, tube (12–)15–19(–22) mm, yellow, lobes (5–)9–12(–16) mm, rounded or obtuse, white, creamy, or pale yellow; stamens usually well exserted, filaments 23–29(–32) mm, occasionally with some glandular trichomes, sericeous at point of insertion for (3–)4–5(–7) mm, adnate to corolla tube for 3–4 mm, anthers 2–3 mm; ovary glabrous, style (17–)23–28(–34) mm, well exserted, divided, branches (2–)4–5(–7) mm, stigma capitate, ovary disc present. Fruit 14–23 × 14–25 × 9–20 mm, schizocarpous, evidently and strongly 4-angled pyriform, brown, gynobase (2–)3–4 mm, carpophore 9–12(–14) mm, exocarp thin, mesocarp spongiose, dry, pyrenes 5–6(–7) × 3(–5) × 2–3(–4) mm, shape 1/4 of a sphere or 1/2 of a cylinder, abaxial surface with 3 to 5 inconspicuous lamellae, reticulate.

Distribution and habitat. *Bourreria exsucca* is the most frequently encountered South American species, and it is found in dry coastal areas or occasionally in wet forests of Colombia to Venezuela from sea level to elevations up to 500(–2315) m. It is geographically isolated from the other schizocarpous species (*B. bolivarensis* in South America; *B. quirosii* Standl., *B. andrieuxii* (DC.) Hemsl., and *B. pulchra* (Millspaugh) Millspaugh in Central America).

IUCN Red List category. Although this species has an AOO below the threshold for consideration as VU, it is widespread and has a large EOO. It also seems to occur in disturbed habitats and is hence best provisionally assigned to LC (IUCN, 2001).

Notes. The species is highly variable in size (leaves, corollas, fruits), shape (leaves), and indument (leaves, inflorescences), but it is distinct from the

other South American species by the combination of the large flowers, with a long tube, and the schizocarpous fruits.

Bourreria exsucca was first described as *Rhamnus cumanensis*, but Schulz's (1911) transfer to *Bourreria* was illegitimate, because Gürke (1893) had already used the combination *B. cumanensis* (DC.) Gürke, which is a heterotypic synonym of *Tournefortia hirsutissima* L. (Miller, 1989).

Pehr Löfling (1729–1756), an ambitious Linnaean student, collected plants with Benito Paltor in Venezuela in 1754. He described *Rhamnus cumanensis*, presumably based on material he collected in Cumaná and near the Franciscan mission in Píritu at Mt. Unare in Sucre. Löfling's surviving collections were deposited at LINN after his early death in 1756, but some of his collections, including those of this species, appear to be among those lost when a ship sank (Stafleu, 1971). In his catalogue of the Linnaean herbarium, Jackson (1912) includes the name *R. cumanensis* in italics, indicating that he was unable to locate a specimen. *Johnston 86* (GH; Fig. 2B) is selected here as a neotype of *Bourreria cumanensis*, since it was collected close to the type locality specified in the protologue, where only one species of *Bourreria* occurs.

Bourreria exsucca was first described by Nicolaus Joseph von Jacquin (1729–1817) in 1763 but without clear designation of a type specimen. D'Arcy (1970) indicates that types of Jacquin names were most frequently found among collections at BM, LINN, MA, and W, but no Jacquin collections were located in these holdings. Jacquin illustrated many plants in the field, from which the Austrian artist Joseph Wagner (1706–1780) produced copper engravings (Stafleu in Jacquin, 1971). Jacquin may have never collected physical material for *B. exsucca*. However, the information available from the type is of limited utility, although the drawings of a flower and a fruit are consistent with Jacquin's protologue, so *Zarucchi & Cuadros 3951* (MO) is selected as an epitype for *B. exsucca*. It was collected near the type locality (Cartagena), where only one species of *Bourreria* occurs, and it helps clarify the morphological circumscription of the species.

Additional specimens examined. COLOMBIA. **Atlántico:** near Baranquilla, *Dugand 429* (F); betw. Ponadera & Santa Rita, *Dugand 686* (F). **Bolívar:** Cartagena, Isla Barú, *Gentry & Cuadros 17619* (MO); Manga Island, Cartagena, *Killip & Smith 14005* (GH), *Killip & Smith 14014* (GH); La Popa, near Cartagena, *Killip & Smith 14053* (GH); Cartagena, *Zarucchi & Cuadros 3928* (MO). **Guajira:** rd. betw. Riohacha & Cuestecitas, *Cuadros 2380* (COL, MO). **Magdalena:** El Callao, *Cuatrecasas & Castaneda 24943* (COL, F); N of La Paz, *Haught 3874* (F, GH, S); near Riohacha, *Haught 4415* (COL, GH, P). **Sucre:** rd. betw. Colosó & Chalan, Ovejas, *Cuadros & Gentry 4668* (JBGP, MO). TRINIDAD AND TOBAGO. **Chacachacare Island:** *Comeau & Johnson 1992* (BM). VENEZUELA. **Anzoátegui:** Bahía de Mochima & Puerto La Cruz, *Aristeguieta & Agostini 5571* (MO); Bruzual, *Castillo & Franca 2969* (MO), Hwy. 9, Km 262, E of Barcelona, *Croat 53926* (MO); Guanta, *Curran & Haman 1233* (GH); Sotillo, *Steyermark, Espinoza & Manara 107723* (MO). **Aragua:** E of Tejerías, *Steyermark 56883* (F). **Distrito Federal:** Vargas, *Castillo 2159* (MO), *Castillo 2488* (MO); old rd. La Guaira–Caracas, *Plowman 7653* (F); Caracas, *Williams 12258* (F, GH, K, U); Silla de Caracas, *Williams 12358* (F, GH). **Falcón:** La Ciénaga, *Breteler 4379* (B, F, M, MA, MO, P); La Vela de Coro, *Curran & Haman 426A* (GH); Paraguana Peninsula, *Curran & Haman 589* (GH); near Pueblo Zazárida, *Liesner & Gonzáles 6024* (MO). **Guárico:** Altagracia, *Aristeguieta 6079* (MO); 6 km S of Ortiz, *Bunting 2206* (GH); 3 km W of Paso Real, *Davidse 4195* (MO); 1 km from Est. C.A.N.T.V., *Puebla 24* (MO, VEN); Roscio, *Rodríguez 43* (F). **Lara:** Barquisimeto, *Alston 6361* (F, GH), *Pittier 13* (GH); 1 km N of Cabudare, *Meijer & Burand 6* (MO); 20 km SW of Barquisimeto, *Steyermark 56830* (F, GH). **Miranda:** near Tovar, *Fendler 920* (GH); Santa Teresa, *Johnson 3638-85* (MO). **Monagas:** Caicara de Maturín, *Cardozo 512* (F, MY). **Nueva Esparta:** Isla de Margarita, *Billiet & Jadin 7973* (MO), *Miller & Johnston 10* (BM, F, GH, MO), *Steyermark et al. 130918* (MO), *Sugden 1299* (MO). **Sucre:** Golfo de Cariaco, *Billiet & Jadin 7952* (MO); Cristobal Colon, *Broadway 468* (GH); Río Caribe, *Curran & Haman 1268* (GH); Las Piedras, *Hahn & Grifo 3371* (MO); Peninsula de Araya, *Liesner & Gonzáles 11977* (MO, WIS); Peninsula de Paria, *Milliken et al. 123* (MO); Cabo Blanco, *Pittier 10209* (GH); Ortiz, *Roberston & Austin 189* (MO); betw. Saucedo & Cariaco, *Rojas 884* (F); Isla Caracas del Este, *Steyermark et al. 108312* (MO); Peninsula de Araya, *Trujillo & Ponce 18639* (MO). **Testigos Islands:** s. loc., *Ferry 1909* (F). **Trujillo:** La Vera, betw. Valera & S. Antonio vía Barquisimeto, *Gaviria 487* (M). **Yaracuy:** 3 km W of Yaritagna, *Gentry & Puig-Ross 14227* (MO). **Zulia:** Mara, *Carnevali & Lobo 679* (MO), *Steyermark et al. 122987* (MO), *Steyermark et al. 123038* (MO), Maracaibo Bot. Garden, *Gentry 41256* (MO).

5. Bourreria mollis Standl., Trop. Woods 8: 5–6. 1926. TYPE: Belize. s. loc., s.d. (fl), *H. W. Winzerling III–12* (holotype, US 1266030!; isotypes, F not seen, GH 94385!, NY!).

Bourreria oxyphylla Standl., Trop. Woods 16: 40–41. 1928. Syn. nov. TYPE: Belize. Cayo: San José, Nov. 1927 (fl), *J. B. Aitken 4* (holotype, F 572622!; isotype, K!).

Bourreria wagneri Standl. in Yunck., Field Mus. Nat. Hist., Bot. Ser. 9: 328. 1940. TYPE: Honduras. Atlántida: La Ceiba, 23 July 1938 (fl, fr), *T. G. Yuncker, J. M. Koepper & K. A. Wagner 8608* (holotype, F 941533!; isotypes, BM 85866!, GH 94393!, MO 1194412!, NY 334954!, NY 334955!, US 1747931!).

Trees, to 12 m tall, glabrous, lenticels absent. Leaves alternate, blade 3.5–10.0 × 1.5–5.0 cm, elliptic to obovate, occasionally asymmetric, apex acuminate, apiculate, rarely rounded or retuse, base acute, frequently asymmetric or decurrent, texture membranaceous to coriaceous, primary vein promi-

nent or rarely rather smooth, secondary veins 6 to 7, tertiary veins reticulate or sometimes percurrent, both surfaces glabrous; stipules absent; petioles 0.3–1.3 cm, slender, canaliculate on adaxial surface or rarely rather smooth, glabrous. Inflorescence 5–8.5 (–14) cm, terminal, comprising up to 50 flowers (or sometimes more), fertile branching sympodial, branches slender, glabrous, bracts frequent, leafy, bracteoles mostly absent. Flowers with pedicles to 3 mm, buds obovoid, slightly apiculate; calyx 5–6 mm, campanulate, coriaceous, glabrous or with few scattered trichomes outside, tomentose at tips inside, usually regularly divided, but irregularly (3 + 2) almost to base in fruit, lobes 5, 1–2 mm, acute, base narrowly cylindrical for 1–2 mm; corolla 10–12 mm, funnel-shaped, membranaceous, glabrous on both surfaces, white, tube 3–5 mm, tubular, sometimes broadened at base, lobes 5, 4–6 mm, rounded or obtuse; stamens well exserted, filaments (5–)8–9 mm, glabrous, adnate to corolla tube for (1–)3–4 mm, anthers 1–2 mm; ovary glabrous, style 8–9 mm, well exserted, divided, branches 3–4 mm, stigma capitate, ovary disc present. Fruits 7–9 × 8–9 × 7–9 mm, drupaceous, depressed ovoid, exocarp thin, mesocarp succulent, voluminous, pyrenes ca. 7 × 4 × 4 mm, shape 1/4 of a sphere, abaxial surface with 4 to 5 conspicuous lamellae and additionally with some vertical ridges.

Distribution and habitat. *Bourreria mollis* is highly variable and occurs mainly in Mexico, Belize, and Guatemala, but a few collections are found in Honduras, Panama, and Colombia.

IUCN Red List category. *Bourreria mollis* is a Central American species with a distribution that barely extends into South America. It occurs in scattered populations and is only rarely collected at the southern end of its distribution. However, it is very common and abundant in Belize and the Yucatán Peninsula of Mexico. The EOO of *B. mollis* is large, and it is provisionally assigned to LC, although its AOO is below the threshold for VU (IUCN, 2001).

Notes. *Bourreria mollis* is most closely related to a group of Caribbean species comprising *B. baccata* and its relatives (here referred to as *B. baccata* species group). Flowers and fruits of *B. mollis* are similar to those of other species of the *B. baccata* species group, but the species can be readily distinguished by leaf morphology. *Bourreria baccata* has broader leaves, with mostly rounded or retuse apices borne on robust petioles. *Bourreria mollis* has leaves that are narrower, with acuminate or apiculate apices borne on most frequently slender petioles. Furthermore, the base of the calyx is evidently constricted in *B. mollis*. This character is otherwise rarely found in *Bourreria*.

Additional specimens examined. COLOMBIA. **Antioquia:** Caucasia, *Brant & Escobar 1234* (HUA, MO), *Callejas et al. 4435* (F, MA, MO).

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ERAGROSTIS (POACEAE: CHLORIDOIDEAE: ERAGROSTIDEAE: ERAGROSTIDINAE) OF PERU[1]

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Abstract

A taxonomic treatment of *Eragrostis* Wolf for Peru is given. Twenty-three species and three subspecies of *Eragrostis* are recognized in the study area. Fifteen species of *Eragrostis* are native to Peru, and eight are adventive or introduced. *Eragrostis attenuata* Hitchc., *E. pilgeri* Fedde, and *E. magna* Hitchc. are endemic to Peru. *Eragrostis lurida* subsp. *contracta* (Pilg.) P. M. Peterson & Sánchez Vega, native to Ecuador and Peru, and *E. pilgeri* subsp. *ancashensis* (P. M. Peterson, Refulio & Tovar) P. M. Peterson & Sánchez Vega, native to Peru, are new combinations. Keys for determining the species, descriptions, distributions, specimens studied, illustrations, synonymies, and a brief discussion of caryopsis morphology for all native and adventive species of *Eragrostis* in Peru are provided. The names *E. andicola* R. E. Fr., *E. contracta* Pilg., *E. tristis* Jedwabn., and *Poa mexicana* Hornem. are lectotypified.

Resumen

Se presenta un estudio taxonómico de *Eragrostis* Wolf para Perú. Veintitrés especies y tres subespecies de *Eragrostis* se reconocen en el área de estudio. Quince especies de *Eragrostis* son nativas de Perú y ocho son advenedizas o introducidos. *Eragrostis attenuata* Hitchc., *E. pilgeri* Fedde y *E. magna* Hitchc. son endémicos de Perú. *Eragrostis lurida* subsp. *contracta* (Pilg.) P. M. Peterson y Sánchez Vega native de Ecuador y Perú, y *E. pilgeri* subsp. *ancashensis* (P. M. Peterson, Refulio y Tovar) P. M. Peterson y Sánchez Vega native de Perú son combinaciones nuevas. Se incluyen claves para determinar las especies, descripciones, distribuciones, los especímenes estudiados, las ilustraciones, las sinonimias, y una breve discusión de la morfología de la cariopsis para todas las especies nativas y advenedizas de *Eragrostis* en Perú. Los nombres *E. andicola* R. E. Fr., *E. contracta* Pilg., *E. tristis* Jedwabn. y *Poa mexicana* Hornem. fueron lectotipificados.

Key words: Chloridoideae, Eragrostideae, Eragrostidinae, *Eragrostis*, Gramineae, Peru, Poaceae.

Peru is a tropical country located in the central and western part of South America between 0° and 18° south latitude. The central Andes or backbone of Peru are important in the formation and delineation of three great regions: coast, mountain, and forest. The coastal region next to the Pacific Ocean is an extremely xeric, narrow, sandy plain that rises abruptly to the east. This region is occasionally transected by river valleys, and the climate is very mild and warm. The mountain region or "cordillera de Andes" consists of high peaks and extensive high plains (altiplano) that are sometimes dissected by deep, narrow valleys. The climate in this region can be very cold, and it is not uncommon to have snow above 3800 m. The tropical forest region east of the cordillera extends into the Amazonian river basin where there are high levels of biodiversity associated with many different habitats. The climate here is very warm and humid with high levels of precipitation, and there are great rivers like the Río Marañon and the Río Ucayali that feed into the Río Amazonas.

The Poaceae are a diverse family that occupies a myriad of terrestrial habitats in Peru, with the exception of the permanent snow fields. The taxonomic knowledge of this family in Peru is incomplete, and it is still possible to encounter novel species. Hitchcock (1927), in his treatment of the grasses of Ecuador, Peru, and Bolivia, recognized 27 species of *Eragrostis* Wolf, 16 of these being reported in Peru. Standley (1936), in his *Flora of Perú*, recorded 19 species. More recently, Tovar (1993) produced an integral study of the Peruvian grasses, including 21

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species of *Eragrostis*. Brako and Zarucchi (1993) list 29 species as being present in Peru; however, many of these are not verified or are synonyms of names in current use. All of these treatments are out of date, and there have been many new reports of grasses from Peru. Because *Eragrostis* is a large genus and is easily dispersed, a modern investigation was undertaken to more clearly understand its biogeography and systematics. We report 23 species and three subspecies of *Eragrostis* in Peru. Two of the subspecies are new combinations.

Eragrostis is a large genus of approximately 350 species occurring in tropical, subtropical, and warm temperate regions throughout the world (Clayton & Renvoize, 1986; Watson & Dallwitz, 1992; Peterson et al., 1995, 1997, 2001). There are 111 species of *Eragrostis* recorded in North, Central, and South America, 67 native in South America (Peterson et al., 2001, 2007; Peterson, 2003). The genus is characterized by having many-flowered spikelets where the disarticulation of the lemma and palea occurs separately, lemmas that are usually 3-nerved and unawned, longitudinally bowed-out paleae with ciliolate keels, paniculate inflorescences, and leaves with ciliate ligules (Peterson et al., 1997). Most species of *Eragrostis* occupy open habitats with poor soils, and many occur in disturbed localities (Clayton & Renvoize, 1986; Van den Borre & Watson, 1994).

All species of *Eragrostis* that have been examined anatomically exhibit "Kranz" or C_4 leaf anatomy (except *E. walteri* Pilg. from South Africa, which exhibits C_3 leaf anatomy; see Ellis, 1984), and species have either chloridoid bicellular microhairs (with the broad, short terminal cell the same thickness as the basal cell) or panicoid bicellular microhairs (with a long, thin-walled terminal cell) (Amarasinghe & Watson, 1990). Apparently, three C_4 biochemical types exist in *Eragrostis*: NAD-ME (nicotinamide adenine dinucleotide co-factor malic enzyme), PCK (phosphoenolpyruvate carboxykinase), and intermediates (Prendergast et al., 1986).

The classification of the tribe Eragrostideae has been problematic, primarily because no one has been able to define this group and select diagnostic characters that exclusively delimit this tribe from other tribes in the Chloridoideae (Hilu & Alice, 2000, 2001; Van den Borre & Watson, 1997, 2000). Historic accounts of the ever-changing opinions of systematists on the classification of the Eragrostideae can be found in Peterson et al. (1995) and Van den Borre and Watson (1994). Peterson et al. (1997) recognized 38 Eragrostideae genera occurring in the New World, then later placed many of these same genera in the tribe Cynodonteae without delineating an Eragrostideae (Peterson et al., 2001). Based on results of a DNA sequence study of the Chloridoideae (Columbus et al., 2007), Peterson et al. (2007) has proposed a completely new classification of the New World Chloridoideae. Here, the Eragrostideae are narrowly interpreted to include only three small subtribes (Cotteneinae, Uniolinae, and Eragrostidinae) that diverge as a clade at the base of the chloridoids (Peterson et al., 2007). Character trends in the Eragrostideae include spikelets with many florets, lemmas with 3 to 13 nerves, and many species adapted to xeric habitats.

The subtribe Eragrostidinae as treated here includes three genera: *Cladoraphis cyperoides* (Thunb.) S. M. Phillips, introduced from Africa; *Eragrostis* with 112 species (86 native New World spp.); and *Steirachne* Ekman with two species (Peterson, 2003; Peterson et al., 2007). The Eragrostidinae are characterized by having hairy or glabrous culm nodes; hairy or glabrous rachillas; entire lemma apices that are awnless, mucronate, or short-awned (only in the latter two genera); glabrous or scabrous lemmas that are (1)3(5)-nerved; and short basal microhair cells (15–75 μm) on the abaxial epidermis of the leaf blade.

Recent systematic treatments of *Eragrostis* from Argentina (Nicora, 1998), Australia (Lazarides, 1997), Bolivia (Renvoize, 1998), Brazil (Boechat & Longhi-Wagner, 2000, 2001), Ecuador (Lægaard & Laegaard & Peterson, 2001), Guianas (Judziewicz, 1991), Malesia (Veldkamp, 2002), Mesoamerica (Davidse, 1994), Mexico (Beetle et al., 1991; Peterson & Valdés Reyna, 2005), the United States and Canada (Peterson, 2003), and Zambesiaca (Cope, 1998) have given us a good understanding of the species limits and their distribution. Based on nuclear and plastid DNA sequences, Ingram and Doyle (2003, 2004, 2007) tested the monophyly of *Eragrostis* and found that, with the inclusion of *Acamptoclados* Nash (*E. sessilispica* Buckley), *Diandrochloa* De Winter, *Neeragrostis* Bush, and *Pogonarthria* Stapf, the genus is indeed monophyletic. However, only 37 species of *Eragrostis* were included in their analysis, so any infrageneric interpretations were beyond the scope of their work. What is lacking is a definitive treatment of the infrageneric classification of the entire genus. Based on spikelet disarticulation, Clayton (1974) and Clayton and Renvoize (1986) have arrived at a first approximation to natural groups and have presented a key to sections *Psilantha* (K. Koch) Tzvelev, *Eragrostis*, *Lappula* Stapf, and *Platystachya* Benth. In most native New World species, the lemmas fall acropetally (from the base toward the apex) from the persistent rachilla, with the paleae persistent on the rachilla. The other major pattern of spikelet disarticulation in *Eragrostis* is when the lemmas fall basipetally (from the apex toward the base). Van den

Borre and Watson (1994) investigated 53 species of *Eragrostis* and found that anatomical characters, among others, support the recognition of two distinct groups: subgenus *Eragrostis* and subgenus *Caesiae* Van den Borre & L. Watson. The most comprehensive attempt so far is Lazarides' (1997) treatment of the Australian *Eragrostis*, in which he recognized six groups primarily based on spikelet disarticulation. Lazarides (1997) correlates his classification with Van den Borre and Watson (1994), who recognized subgenus *Eragrostis* and subgenus *Psilantha*, and with Amarasinghe and Watson (1990), who investigated microhair morphology within the genus. Cope's (1998) informal treatment of *Eragrostis* for the flora of Zambesiaca is also comprehensive since he delineates nine species groups based on panicle, lemma, and palea morphology in addition to spikelet disarticulation. Based on caryopsis morphology, Boechat and Longhi-Wagner (2003) placed 49 of the 53 species of *Eragrostis* that occur in Brazil into the following six groups: smooth-walled (six species), medianly reticulate (14 species), roughly reticulate (10 species), finely reticulate (nine species), alveolate (six species), and striate (four species). Overall morphological features led Peterson and Valdés Reyna (2005) to recognize the following four hypothesized lineages within 26 species of *Eragrostis* from northeastern Mexico: an Old World group, the *E. intermedia* Hitchc. complex, the *E. pectinacea* complex, and the *E. spectabilis* (Pursh) Steud.–*E. secundiflora* J. Presl group.

Our taxonomic treatment contains a key for determining species, descriptions, distribution, specimens studied, illustrations, synonymies, and a brief discussion of caryopsis morphology and its usefulness in hypothesizing relationships among the native and adventive species of *Eragrostis* in Peru. This study is based on the examination of herbarium specimens from AMAZ, CPUN, CUZ, HAO, HUT, US, and USM, including the type specimens of most of the species studied.

Taxonomic Treatment

Eragrostis Wolf, Gen. Pl. 23. 1776. TYPE: *Eragrostis minor* Host, Icon. Descr. Gram. Austriac. 4: 15. 1809 (lectotype, designated by Ross, Acta Bot. Neerl. 15: 157. 1966).

Plants annual or perennial; usually synoecious, sometimes dioecious; caespitose, stoloniferous, or rhizomatous; flowering culms (2–)5–150 cm tall, not woody, erect, decumbent, or geniculate, sometimes rooting at the lower nodes, simple or branched; internodes solid or hollow; bud initiation intravaginal, rarely extravaginal. Leaf sheaths open, often with tufts of hairs at the apices, hairs 0.3–9 mm; ligules usually membranous and ciliolate or ciliate, cilia sometimes longer than the membranous base, occasionally of hairs or membranous and non-ciliate; blades flat, folded, or involute. Inflorescences terminal, sometimes also axillary, simple panicles, open to contracted or spike-like, terminal panicles usually exceeding the upper leaves; pulvini in the axils of the primary branches glabrous or hairy; branches not spike-like, not disarticulating. Spikelets 1–20 × 0.5–4.5 mm, laterally compressed, with 2 to 40 florets; disarticulation below the fertile florets, sometimes also below the glumes, acropetal with deciduous glumes and lemmas but persistent paleae, or basipetally with the glumes often persistent and the florets usually falling intact; glumes usually shorter than the adjacent lemmas, (1)3(5)-veined, not lobed, apices obtuse to acute, unawned; calluses glabrous or sparsely pubescent; lemmas usually glabrous, obtuse to acute, 3(5)-veined, usually keeled, unawned or mucronate; paleae shorter than the lemmas, longitudinally bowed-out by the caryopses, 2-keeled, keels usually short or long ciliate, intercostal region membranous or hyaline; anthers 2 or 3; ovaries glabrous; styles free to the bases. Cleistogamous spikelets occasionally present, sometimes on the axillary panicles, sometimes on the terminal panicles. Caryopses variously shaped; hilum short; embryo with an epiblast, scutellar tail, and elongated mesocotyl internode (formula P + PF), endosperm hard. Base chromosome number, $x = 10$ (Watson & Dallwitz, 2003).

Comments. In most taxa native to the Western Hemisphere, disarticulation of the spikelet is acropetal (florets first maturing at the base) and the lemmas fall with the caryopses, leaving paleae attached to the rachilla. The common mode of disarticulation for introduced species from Africa and Asia is basipetally, where the florets near the apex mature first.

Etymology. The origin of the name is somewhat obscure. Nathaniel von Wolf (1776), who first named *Eragrostis*, made no statement concerning the origin of its name. Clifford (1996) provides three possible derivations: from "eros" (love) and "Agrostis" (the Greek name for an indeterminate herb); from the Greek "er" (early) and "agrostis" (wild), referring to the fact that some species of *Eragrostis* are early invaders of arable land; or the Greek "eri-" (a prefix meaning "very" or "much"), suggesting that the name means many-flowered "Agrostis." Watson and Dallwitz (2005) indicate that the derivation of *Eragrostis* is "from the Greek 'eros' (love) or 'era' (earth) and 'agrostis' (a grass), probably alluding to the characteristic, earthy (human) female aroma of the inflorescences of many species."

Ecology and geographic distribution. The 23 species of *Eragrostis* in Peru are widely distributed among the coast, mountain, and forest regions. The annual species are more conspicuous along the sandy plain of the coast, the interior valleys of the western slopes, and in the dry Andean valleys at lower elevations. Along the north coast, *E. tenella* (L.) P. Beauv. ex Roem. & Schult., *E. ciliaris* (L.) R. Br., and *E. cilianensis* (All.) Vignolo ex Janch. form part of the xerophytic vegetation that flowers between January and March. The lomas vegetation to the south of 8 south latitude contains *E. attenuata* Hitchc., *E. peruviana* (Jacq.) Trin., and *E. weberbaueri* Pilg. and generally flowers between June and October. In the mountains, species of *Eragrostis* are an integral component of natural, high-elevation ecosystems. *Eragrostis nigricans* (Kunth) Steud. and *E. pilosa* (L.) P. Beauv. are usually found growing along margins of cultivated fields, while many other species inhabit edges of roads and trails. *Eragrostis lurida* J. Presl, *E. magna* Hitchc., *E. pastoensis* (Kunth) Trin., *E. pilgeri* Fedde, and *E. weberbaueri* are principally found in the altiplano and puna of southern Peru, and disappear in the jalca or páramo in the north. In this mountainous region of central and southern Peru, there is a greater number of perennial, native species of *Eragrostis*. *Eragrostis hypnoides* (Lam.) Britton, Sterns & Poggenb. is the only species commonly found along the coast and in the Amazonian forest in areas that are periodically flooded (i.e., margins of rivers).

Caryopsis morphology. The caryopsis contains many morphological features that are important aids in the identification of species, and this information can be used to infer hypothetical relationships among the grasses (Colbry, 1957; Terrell & Peterson, 1993; Boechat & Longhi-Wagner, 2003). In *Eragrostis*, grains can be terete, subterete, or rectangular or trigonous in cross section and are sometimes compressed either dorsally (on a plane with the emryo) or laterally (Figs. 1–6). The shape of the grain can vary from spherical to much longer than broad (ellipsoid, obovoid, ovoid, rectangular-prismatic, etc.). The embryo is located on the dorsal (abaxial) surface of the grain, and the hilum is the tiny scar left from the attachment of the funiculus found near the base on the ventral surface. The ventral (adaxial) surface can be rounded, flattened, or sometimes have a sulcus or groove running longitudinally along the body. The surface of the grain can be smooth to variously sculptured and is often striate to reticulate. In the grasses, the surface or pericarp of the grain is almost always adnate, i.e., a true caryopsis; however, a few species of *Eragrostis* (see *E. japonica* (Thunb.) Trin.) can have loose pericarps that shed when the grain is moistened. The color of the grain can vary in the Peruvian *Eragrostis* from light brownish or whitish to reddish brown, and the grains can be translucent to opaque.

The most common caryopsis type found in Peruvian *Eragrostis* is the rectangular-prismatic to irregularly triangular, laterally flattened, striate, reticulate-walled, and ventrally grooved grain. This is found predominately in species that occupy the altiplano such as *E. lurida*, *E. magna*, *E. pastoensis*, and *E. pilgeri*, as well as in *E. andicola* R. E. Fr., *E. lugens* Nees, *E. soratensis*, *E. mexicana* (Hornem.) Link, *E. nigricans*, and *E. tenuifolia* (A. Rich.) Hochst. ex Steud. (of African origins). *Eragrostis pectinacea* (Michx.) Nees and *E. pilosa* are similar to the last group, although these two species have grains that are striate only and rectangular in cross section, so they are at least flattened ventrally but do not have a groove. *Eragrostis maypurensis* (Kunth) Steud. is unique in possessing rhomboid reticulations without striations. *Eragrostis tenella*, *E. atrovirens* (Desf.) Trin. ex Steud., *E. attenuata*, *E. cilianensis*, *E. ciliaris*, *E. curvula* (Schrad.) Nees, *E. hypnoides*, *E. japonica*, *E. maypurensis*, *E. peruviana*, and *E. weberbaueri* are all circular or elliptical to ovate/obovate in cross section. *Eragrostis tenella*, *E. attenuata*, *E. ciliaris*, *E. curvula*, and *E. japonica* are striate or smooth with no evidence of reticulations. *Eragrostis ciliaris*, *E. japonica*, *E. pilosa*, and *E. curvula* all have somewhat dorsally flattened caryopses. *Eragrostis curvula*, in addition to being strongly dorsally flattened, can sometimes possess a shallow and broad ventral groove.

Key to the Species of *Eragrostis* in Peru

1a. Annuals, caespitose, stoloniferous or mat-forming to geniculate, without innovations at the basal nodes.
  2a. Palea keels prominently ciliate, the cilia 0.2–0.8 mm long.
    3a. Panicles 1–7 cm wide, open, narrowly ovate; primary branches ascending and spreading 20 –100 from the rachises; spikelets (1–)1.5–2.2 mm long . . . . . 1. *E. tenella*
    3b. Panicles 0.2–1.5(–3) cm wide, cylindrical, spiciform and/or spike-like; primary branches ascending and tightly appressed; spikelets 1.8–4.5 mm long.
      4a. Spikelets 1.8–3.2 mm long; cilia on the palea stiff and pectinate-thickened near base; stamens 2 . . . . . 6. *E. ciliaris*
      4b. Spikelets 3–4.5 mm long; cilia on the palea soft and silky, not pectinate-thickened near base; stamens 3 . . . . . 18. *E. peruviana*

2b. Palea keels smooth or scabrous, the scabridities less than 0.2 mm long.
5a. Plants extensively stoloniferous and mat-forming; panicles 1–3.5 cm long, terminal and axillary; culms (2–)5–12(–20) cm tall . . . . . 8. *E. hypnoides*
5b. Plants not stoloniferous but occasionally mat-forming; panicles (3–)4–40 cm long; culms 8–130 cm tall.
6a. Ligules membranous, glabrous . . . . . 9. *E. japonica*
6b. Ligules membranous and ciliate, with a row of tiny white hairs.
7a. Crateriform glands present on the keels of the lemma, these glands usually present on the keels of the glumes, margins of the blades, sheaths, and below the nodes of the culm . . . . . 5. *E. cilianensis*
7b. Crateriform glands not present on the keels of the lemma, keels of the glumes, and margins of the blades, occasionally present below the nodes of the culm and sheaths.
8a. Caryopses with a shallow or deep ventral groove.
9a. Spikelets not arranged in glomerules, 5- to 11(to 15)-flowered, (4–)5–10(–11) mm long; pedicels appressed to narrowly divergent, stiff . . . . . 14. *E. mexicana*
9b. Spikelets arranged in glomerules, 2- to 4(to 5)-flowered, 2.6–3.8 mm long; pedicels spreading, divaricate and stout . . . . . 15. *E. nigricans*
8b. Caryopses without a ventral groove.
10a. Lower glumes 2–2.6 mm long, usually longer than upper; pedicels 0–1.5 mm long, shorter than the spikelets; lemmas chartaceous, lateral nerves and midnerve evident, usually green; stamens 2; caryopses obovate to circular in cross section . . . . . 13. *E. maypurensis*
10b. Lower glumes 0.3–1.5 mm long, shorter than the upper; pedicels 0.8–10 mm long, longer or shorter than the spikelets; lemmas hyaline to membranous, lateral nerves and midnerve inconspicuous to moderately conspicuous, never green; stamens 3; caryopses rectangular in cross section.
11a. Lower glumes 0.5–1.5 mm long, at least 1/2 as long as the lowest lemmas; paleae persistent; spikelets 1.2–2.5 mm wide; panicle branches solitary or paired at the lowest two nodes; lemmas with moderately conspicuous lateral nerves . . . . . 17. *E. pectinacea*
11b. Lower glumes 0.3–0.6(–0.8) mm long, usually less than 1/2 as long as the lowest lemmas; paleae easily deciduous; panicle branches usually whorled at the lowest two nodes; lemma with inconspicuous lateral nerves . . . . . 20. *E. pilosa*
1b. Plants perennial or biennial, caespitose, forming innovations at the basal nodes.
12a. Panicles 0.2–2 cm wide, contracted, densely flowered, cylindrical and narrowly spicate to spiciform, the primary branches ascending and tightly appressed.
13a. Panicle rachis densely pilose, the hairs not rigid; culm nodes silky pilose, the hairs up to 3 mm long; blades densely silky pilose above and below . . . . . 23. *E. weberbaueri*
13b. Panicle rachis glabrous; culm nodes glabrous or occasionally with a tuft of hairs, the hairs less than 1 mm long; blades glabrous, scabrous, with a few scattered hairs or short pilose but not densely silky.
14a. Panicles 2–5 mm wide; lower glumes 0.8–1 mm long; lemma apex obtuse; caryopses only striate, elliptical in cross section and without a ventral groove; anthers about 0.3 mm long . . . 4. *E. attenuata*
14b. Panicles 5–20 mm wide; lower glumes 1–1.5 mm long; lemma apex acute; caryopses striate and reticulate, rectangular in cross section and usually with a ventral groove or at least flattened ventrally; anthers 0.3–0.6 mm long.
15a. Secondary panicle branches condensed into tightly glomerate lobes of spikelets; pedicels 0.1–1 mm long, scabrous; lemma with conspicuous lateral nerves, lemma 1.6–2.4 mm long; blades scaberulous above without additional hairs . . . . . 11b. *E. lurida* subsp. *contracta*
15b. Secondary panicle branches composed of loosely overlapping spikelets; pedicels 0.5–5 mm long; lemma with inconspicuous lateral nerves, lemma 1.2–2.1 mm long; blades scaberulous above, sometimes with scattered hairs up to 4 mm long . . . . . 16. *E. pastoensis*
12b. Panicles 2–27 cm wide, open, not contracted, the primary branches ascending and spreading to divaricate.
16a. Lemmas 2–3.2 mm long; upper glumes (1.8–)2–3 mm long.
17a. Anthers 0.4–0.7(–0.8) mm long; paleae 1.2–2.4 mm long.
18a. Secondary panicle branches composed of loosely overlapping spikelets; blades with scattered small oblong glands located above the veins; sheaths with papillose-based hairs; pedicels 1.8–5 mm long, divaricate at maturity with a single glandular band near the middle . . . . . 2. *E. andicola*
18b. Secondary panicle branches; blades without small oblong glands located above the veins; sheaths without papillose-based hairs; pedicels 0.1–1 mm, ascending and appressed, without any glandular bands . . . . . 11. *E. lurida*
17b. Anthers 0.8–2 mm; paleae 2–3.1 mm.
19a. Spikelets 1.2–2 mm; caryopses dorsally flattened, smooth to striate, elliptical in cross section, ventral surface with a shallow, broad groove or ungrooved . . . . . 7. *E. curvula*
19b. Spikelets 2–4.5 mm wide; caryopses laterally flattened to rectangular-prismatic, rectangular to triangular in cross section, with a deep ventral groove.

20a. Culms 80–150 cm tall, with short rhizomes near base; spikelets 2–2.4 mm wide; rachillas hairy only near apex, the hairs less than 0.2 mm long; blades 26–60 cm long, 3–7 mm wide . . . . . 12. *E. magna*

20b. Culms 26–84 cm tall, without rhizomes; spikelets (2–)2.2–4.5 mm wide; rachillas hairy along entire length, the hairs 0.4–1.8 mm long; blades (6–)10–22(–26) cm long, 1–2.5(–3) mm wide . . . . . 19. *E. pilgeri*

16b. Lemmas 1.2–2(–2.1) mm long; upper glumes 0.5–2 mm long.

21a. Spikelets 6–12(–19) mm long, 6- to 22-flowered.

22a. Culms (70–)75–130 cm tall; lower glumes 1.2–1.4 mm long; upper glumes 1.4–1.7 mm long; caryopses circular in cross section and not laterally flattened and without a ventral groove; anthers 0.7–0.9 mm long . . . . . 3. *E. atrovirens*

22b. Culms 25–75 cm tall; lower glumes 0.3–0.6 mm long; upper glumes 0.5–1 mm long; caryopses narrowly triangular in cross section, strongly laterally flattened with a deep ventral groove; anthers 0.2–0.4 mm long . . . . . 22. *E. tenuifolia*

21b. Spikelets 2–6 mm long, 2- to 10-flowered.

23a. Panicles 0.5–5(–7) cm wide, narrowly ovate, the primary branches floriferous near base; secondary branches condensed into tightly glomerate lobes of spikelets; pedicels 0.1–1 mm long, ascending and appressed; lemmas with conspicuous lateral nerves . . . . . 11. *E. lurida*

23b. Panicles 2–27 cm wide, open, ovate, the primary branches not floriferous near base; secondary branches composed of loosely overlapping spikelets; pedicels 0.5–6(–7) mm long, appressed to spreading; lemmas with inconspicuous lateral nerves.

24a. Spikelets 0.5–1.1 mm wide; lower glumes 0.6–1 mm long; blades 1–2(–2.2) mm wide, involute, rarely flat . . . . . 10. *E. lugens*

24b. Spikelets 1–1.8 mm wide; lower glumes 1–1.4 mm long; blades 1–4(–7) mm wide, flat or involute.

25a. Culms 30–90 cm tall; blades 5–40(–45) cm long; pedicels mostly appressed; sheaths 3/4 as long as the internodes above . . . . . 16. *E. pastoensis*

25b. Culms 10–40 cm tall; blades 4–10 cm long; pedicels spreading; sheaths longer than the internodes above . . . . . 21. *E. soratensis*

1. Eragrostis tenella (L.) P. Beauv. ex Roem. & Schult., Syst. Veg. 2: 576. 1817. Basionym: *Poa tenella* L., Sp. Pl. 1: 69. 1753. *Megastachya tenella* (L.) Bojer, Hortus Maurit. 369. 1837. TYPE: India. (lectotype, designated by Veldkamp, Blumea 47: 164. 2002, Herb. Linn. 87.33-LINN!; IDC microfiche US!). Figures 6A–D; 7A, B.

Poa amabilis L., Sp. Pl. 68. 1753. *Eragrostis amabilis* (L.) Wight & Arn., Bot. Beechey Voy. 251. 1838, hom. illeg. *Megastachya amabilis* (L.) P. Beauv., Ess. Agrostogr. 74, 167, 173. 1812. *Cynodon amabilis* (L.) Raspail, Ann. Sci. Nat., Bot. 5: 302. 1825. TYPE: Sri Lanka. *Herb. Hermann 2: 59, no. 46* (lectotype, designated by Veldkamp in Cafferty et al., Taxon 19(2): 254. 2000, BM!; IDC microfiche US!).

Poa plumosa Retz., Observ. Bot. 4: 20. 1786. *Eragrostis plumosa* (Retz.) Link, Hort. Berol. 1: 192. 1827. *Eragrostis tenella* var. *plumosa* (Retz.) Stapf, Fl. Brit. India 7(22): 315. 1897 (1896). *Eragrostis amabilis* var. *plumosa* (Retz.) E. G. Camus & A. Camus, Fl. Indo-Chine 7: 557. 1923. TYPE: India. E. Tranquebaria, *König s.n.* (holotype, LD not seen; isotype, K [fragm.]!).

Eragrostis ciliaris var. *patens* Chapm. ex Beal, Grasses N. Amer. 2: 479. 1896. TYPE: U.S.A. Georgia: Wayne Co., Doctortown & Jesup, Sep.–Oct. 1880, *A. H. Curtiss 3193* (holotype, MSC not seen; isotypes, NY!, US 748296!, US 821866!, US 909340!).

Annual, caespitose; culms 5–40(–50) cm tall, erect to spreading, mostly glabrous and occasionally with oblong-glandular areas below the nodes. Leaf sheaths 1/2 as long as the internodes, ciliate at the summit, collar, and along margins of the upper portion, the hairs to 4 mm long, stiff; ligules 0.2–0.3 mm, ciliate; blades 2–8 × 0.2–0.4 cm, flat to involute, glabrous below and scaberulous above, occasionally with a few scattered papillose-based hairs below. Panicles 4–15 × 1–7 cm, open, narrowly ovate, primary branches 0.5–4 cm, sometimes with irregular glandular areas below the branch bases, branches diverging 20°–100° from the rachises; pulvini ciliate or glabrous; pedicels 0.8–4(–7) mm, mostly pendent, drooping to erect. Spikelets (1–)1.5–2.2 × 0.9–1.2 mm, 4- to 8-flowered, ovate to oblong, reddish purple to greenish; disarticulation between the florets with a portion of the rachilla; glumes 0.4–1.1 mm, unequal, ovate, hyaline, keeled, the nerve commonly green, scaberulous along the keel; lower glume 0.4–0.7 mm; upper glume 0.7–1.1 mm; lemmas 0.7–1.1 mm, ovate to broadly oblong, membranous, lateral nerves evident, usually greenish, strongly keeled, scaberulous along keel; apex truncate to obtuse; paleae 0.6–1.1 mm, hyaline, keels ciliate, the cilia 0.3–0.5 mm; apex obtuse to truncate; stamens 3, anthers ca. 0.2 mm, purplish. Caryopses 0.3–0.5 mm, ellipsoid, faintly striate, elliptical to circular in cross section, translucent, light brown. $2n = 20$ (Baquar & Saeed, 1969).

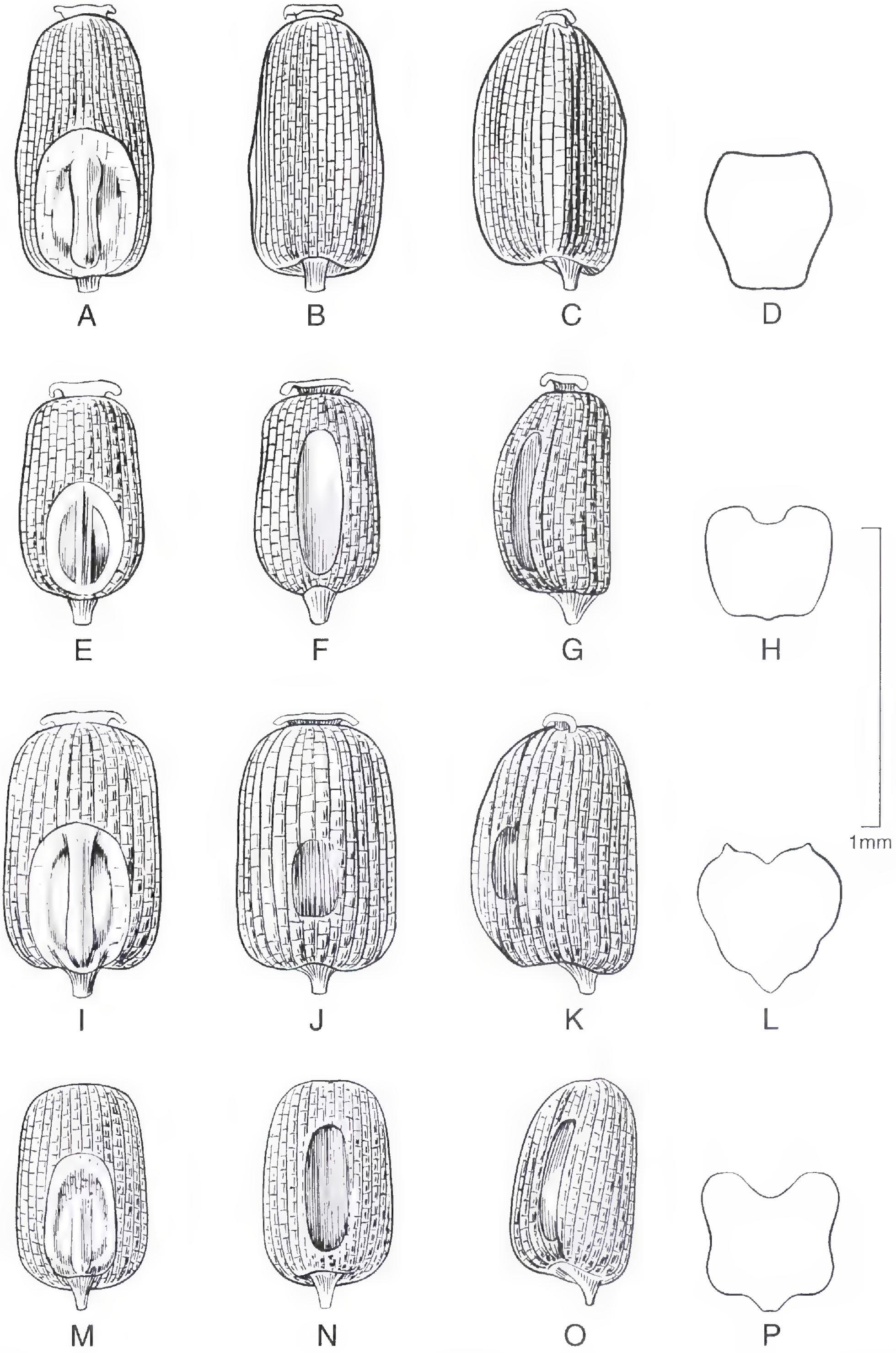

Figure 1. Caryopses of *Eragrostis* species. —A–D. *E. lurida* subsp. *lurida* (*P. M. Peterson & N. F. Refulio-Rodriguez 13993*). —E–H. *E. pastoensis* (*P. M. Peterson & N. F. Refulio-Rodriguez 13982*). —I–L. *E. lugens* (*I. Sánchez Vega 3218*). —M–P. *E. nigricans* (*M. O. Dillon, U. Molau & P. Matekaitis 3258*). Dorsal views (A, E, I, M); ventral views (B, F, J, N); lateral views (C, G, K, O); cross sections (D, H, L, P).

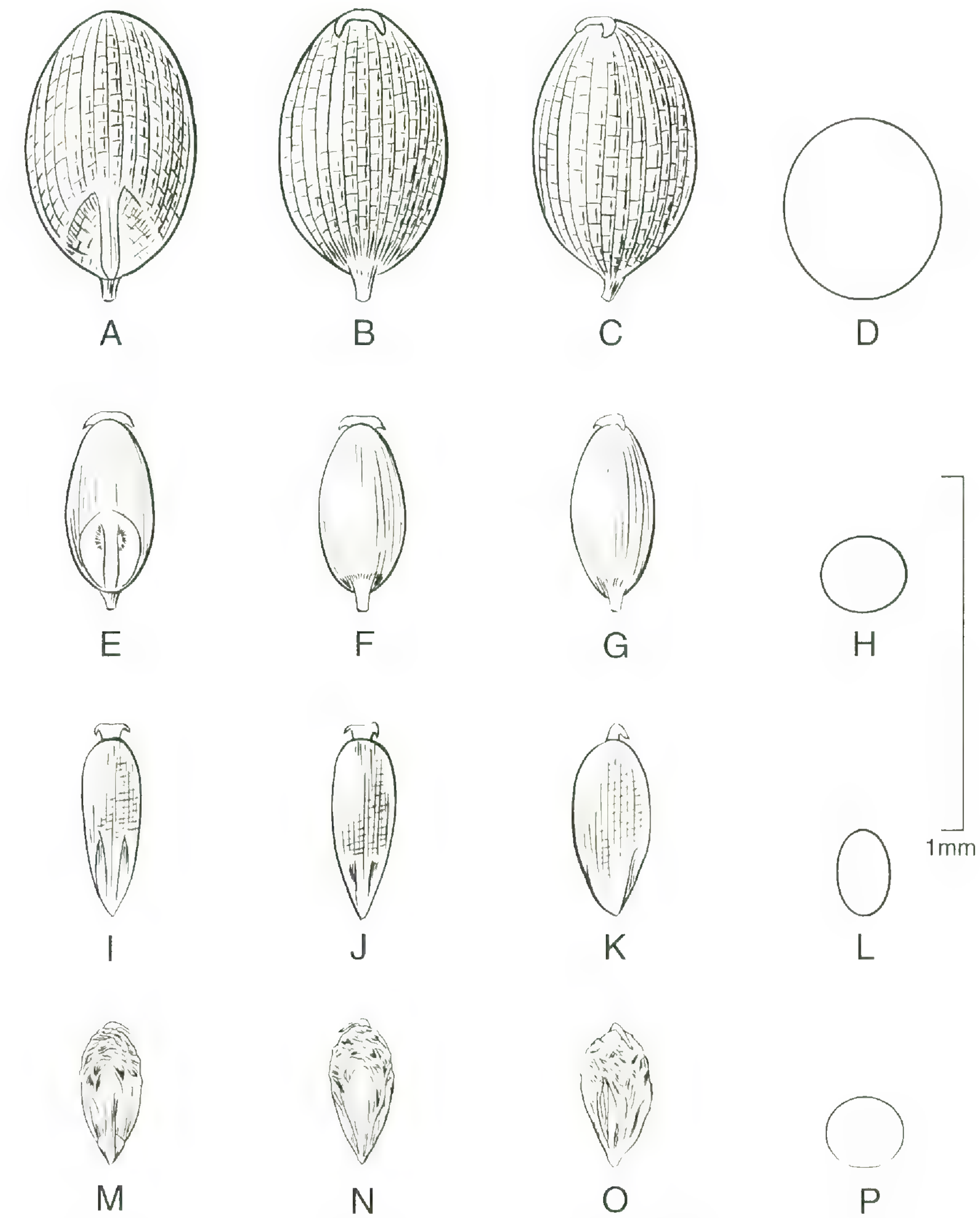

Figure 2. Caryopses of *Eragrostis* species. —A–D. *E. cilianensis* (*R. Ferreyra 6017*). —E–H. *E. ciliaris* (*A. Sagástegui A. 10927*). —I–L. *E. hypnoides* (*A. López M. & A. Sagástegui A. 4023*). —M–P. *E. japonica* (*E. Asplund 14802*). Dorsal views (A, E, I, M); ventral views (B, F, J, N); lateral views (C, G, K, O); cross sections (D, H, L, P).

Distribution and habitat. Native in India; introduced in U.S.A., Mexico, Central America, Caribbean, Brazil, Bolivia, Colombia, Ecuador, Guianas, Paraguay, Peru, and Venezuela (Nicora, 1998; Peterson & Boechat, 2001); occurs in open areas near cultivated fields, dry forests, and along roadsides only in northwestern Peru; 0–1500 m.

Phenology. Flowering October through May.

Specimens examined. PERU. **Amazonas:** Prov. Bagua, Chiriaco–Nazareth, *F. Holle 20575* (USM). **Cajamarca:** Prov. Jaén, 1 km N of Chamayo on Hwy. 5N toward Jaén, *P. M. Peterson & N. F. Refulio-Rodriguez 15056* (US, USM); 6 km W of Hwy. 5N up Río Tabaconas toward Tamborillo, *P. M. Peterson & N. F. Refulio-Rodriguez 15087* (US, USM). **Lambayeque:** Prov. Lambayeque, betw. Chochope & La Ramada, *J. T. Columbus, J. M. Porter & E. H. Roalson 3442* (RSA, US); Prov. Lambayeque, El Porvenir (Olmos), *S. Llatas Q. 1180* (US). **Piura:** Prov. Piura, 15 km entre Piura y Sullana, *I. Sánchez Vega & J. Guevara 6191* (CPUN, US). **Tumbes:** Prov. Contralmirante Villar, SE de Zorritos, *R. Ferreyra 12268* (US).

2. **Eragrostis andicola** R. E. Fr., Nova Acta Regiae Soc. Sci. Upsal., ser. 4, 1(1): 180. 1905. TYPE: Argentina. Prov. Jujuy: Moreno, "in

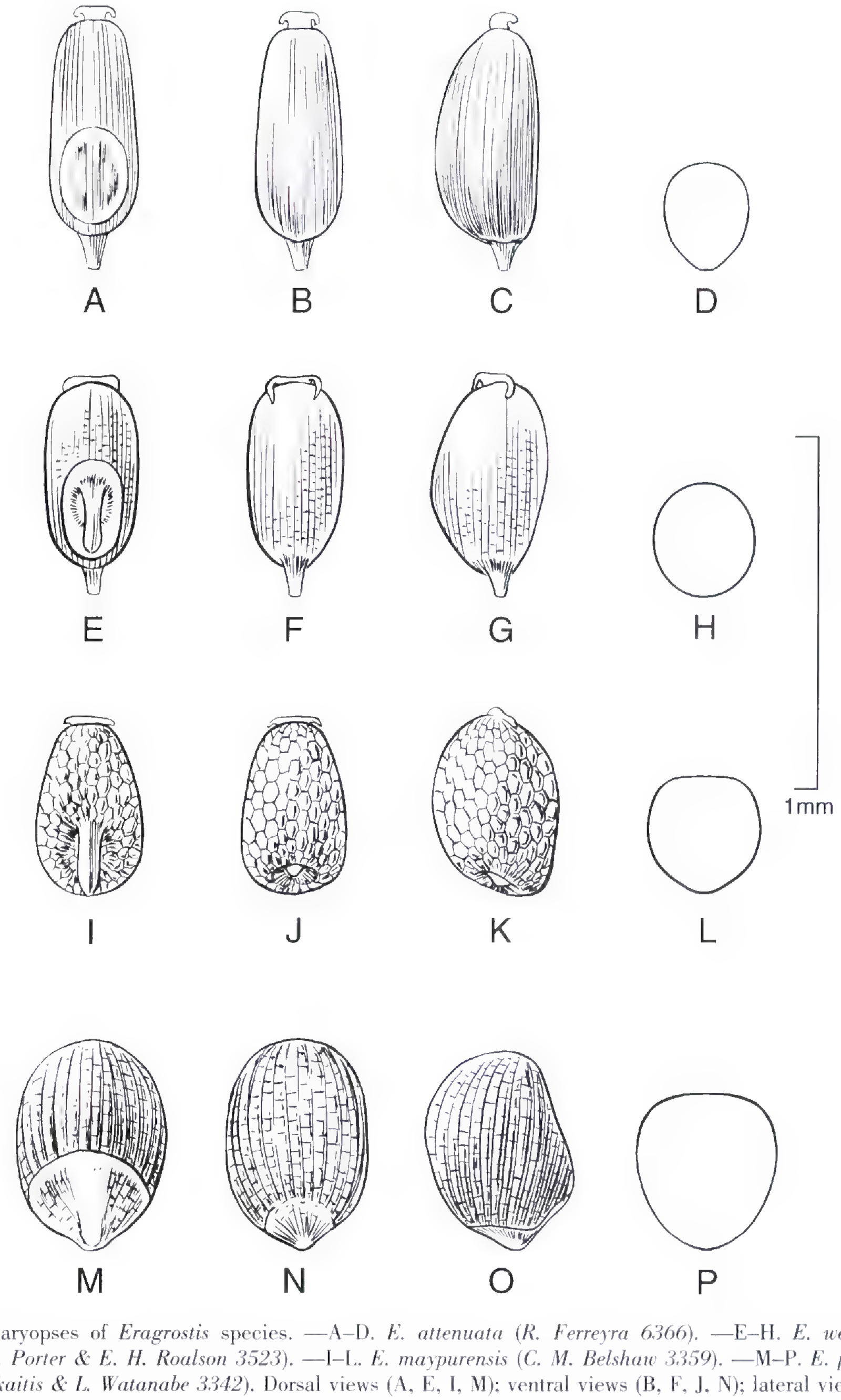

Figure 3. Caryopses of *Eragrostis* species. —A–D. *E. attenuata* (*R. Ferreyra 6366*). —E–H. *E. weberbaueri* (*J. T. Columbus, J. M. Porter & E. H. Roalson 3523*). —I–L. *E. maypurensis* (*C. M. Belshaw 3359*). —M–P. *E. peruviana* (*M. O. Dillon, P. Matekaitis & L. Watanabe 3342*). Dorsal views (A, E, I, M); ventral views (B, F, J, N); lateral views (C, G, K, O); cross sections (D, H, L, P).

montibus saxosis apreicis siccis," 3500–3700 m, 15 Dec. 1901, *R. E. Fries 924* (lectotype, designated here, UPS!; isotypes, BAA!, CORD, US 2891444 [fragm.] ex S!). Figures 4E–H; 8A–C.

Perennial, caespitose with thick roots; culms 13–50 cm tall, erect, mostly glabrous and sometimes with small oblong glands located on the veins, 1 node per culm. Leaf sheaths 1–1 1/2 as long as the internode below, ciliate at the summit, collar, and usually the margins above to pilose throughout, the papillose-based hairs up to 4 mm, stiff; with small oblong glands located on the veins; ligules 1–1.5 mm, ciliate; blades (3–)5–12 × 0.15–0.3 cm, flat to involute, strongly divaricate, with scattered

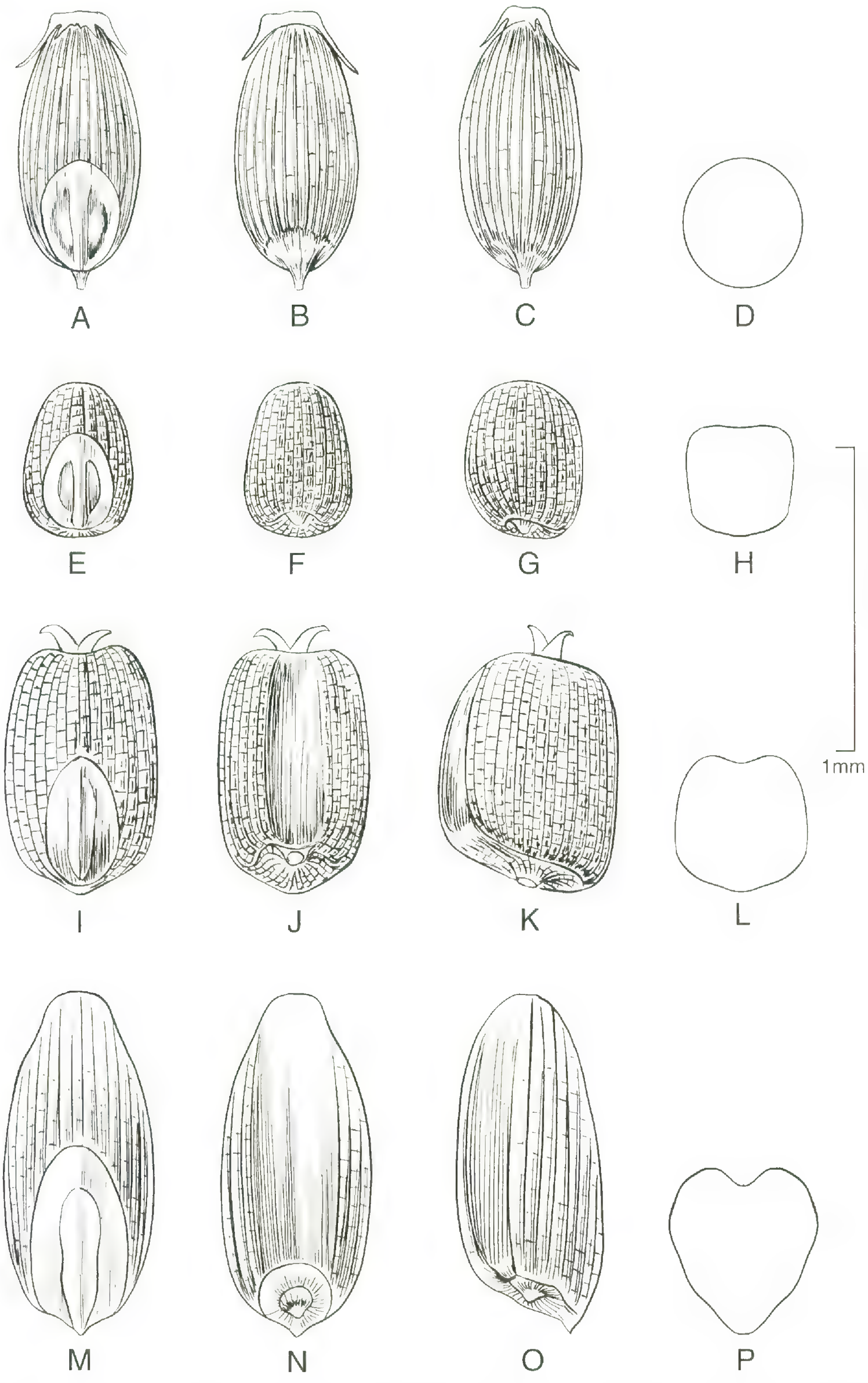

Figure 4. Caryopses of *Eragrostis* species. —A–D. *E. atrovirens* (*I. Sánchez-Vega 9651*). —E–H. *E. andicola* (*J. F. Mabride 3516*). I–L. *E. pilgeri* subsp. *ancashensis* (*P. M. Peterson & N. F. Refulio-Rodriguez 13793*). —M P. *E. pilgeri* subsp. *pilgeri* (*I. Sánchez-Vega & V. Torrel 2380*). Dorsal views (A, E, I, M); ventral views (B, F, J, N); lateral views (C, G, K, O); cross sections (D, H, L, P).

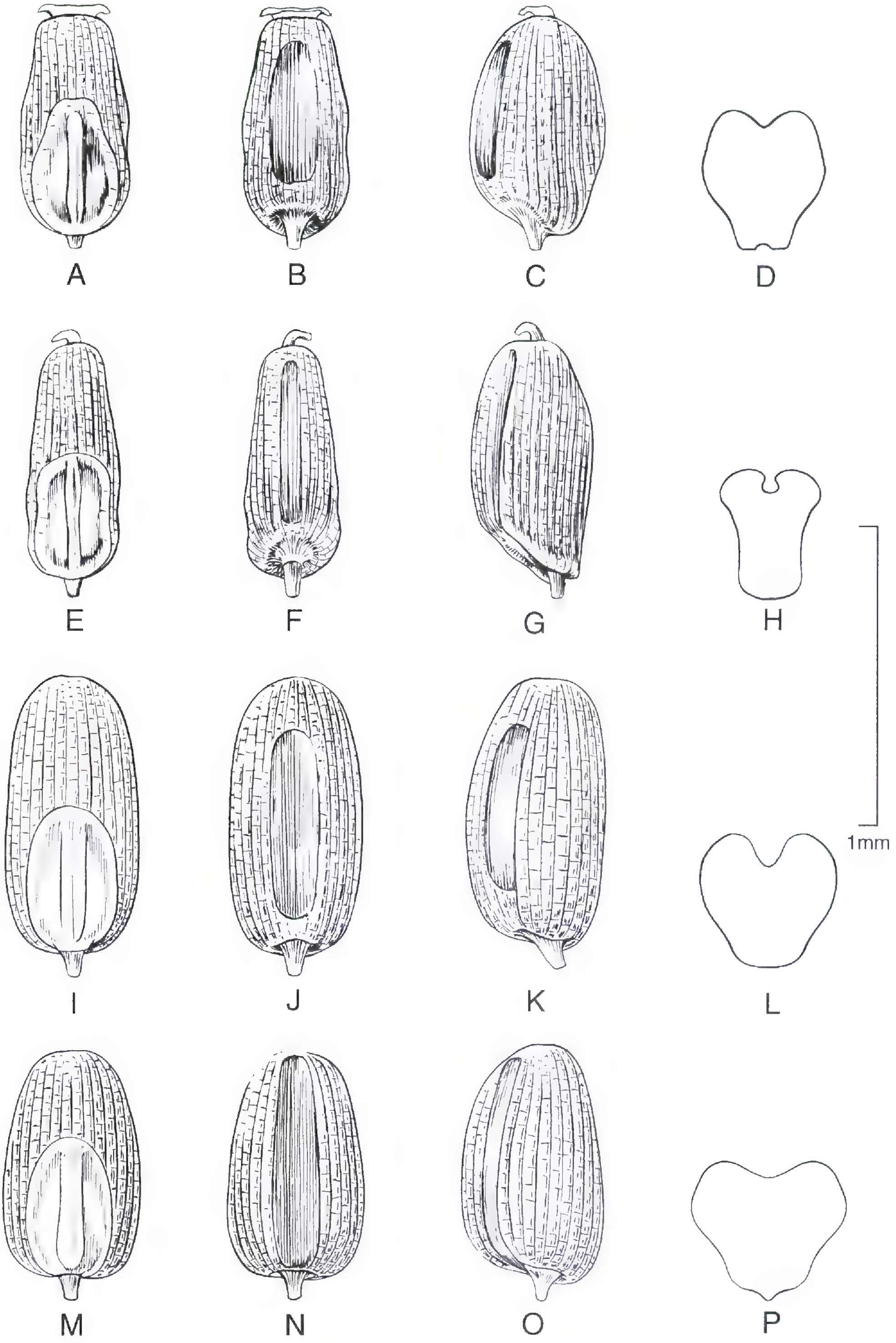

Figure 5. Caryopses of *Eragrostis* species. —A–D. *E. soratensis* (*P. M. Peterson, N. F. Refulio Rodriguez & F. Salvador Pérez 14625*). —E–H. *E. magna* (*J. F. MacBride 4069 & P. M. Peterson & N. F. Refulio-Rodriguez 16515*). —I–L. *E. mexicana* subsp. *mexicana* (*I. Sánchez-Vega 4020*). —M–P. *E. mexicana* subsp. *virescens* (*I. Sánchez-Vega 2301*). Dorsal views (A, E, I, M); ventral views (B, F, J, N); lateral views (C, G, K, O); cross sections (D, H, L, P).

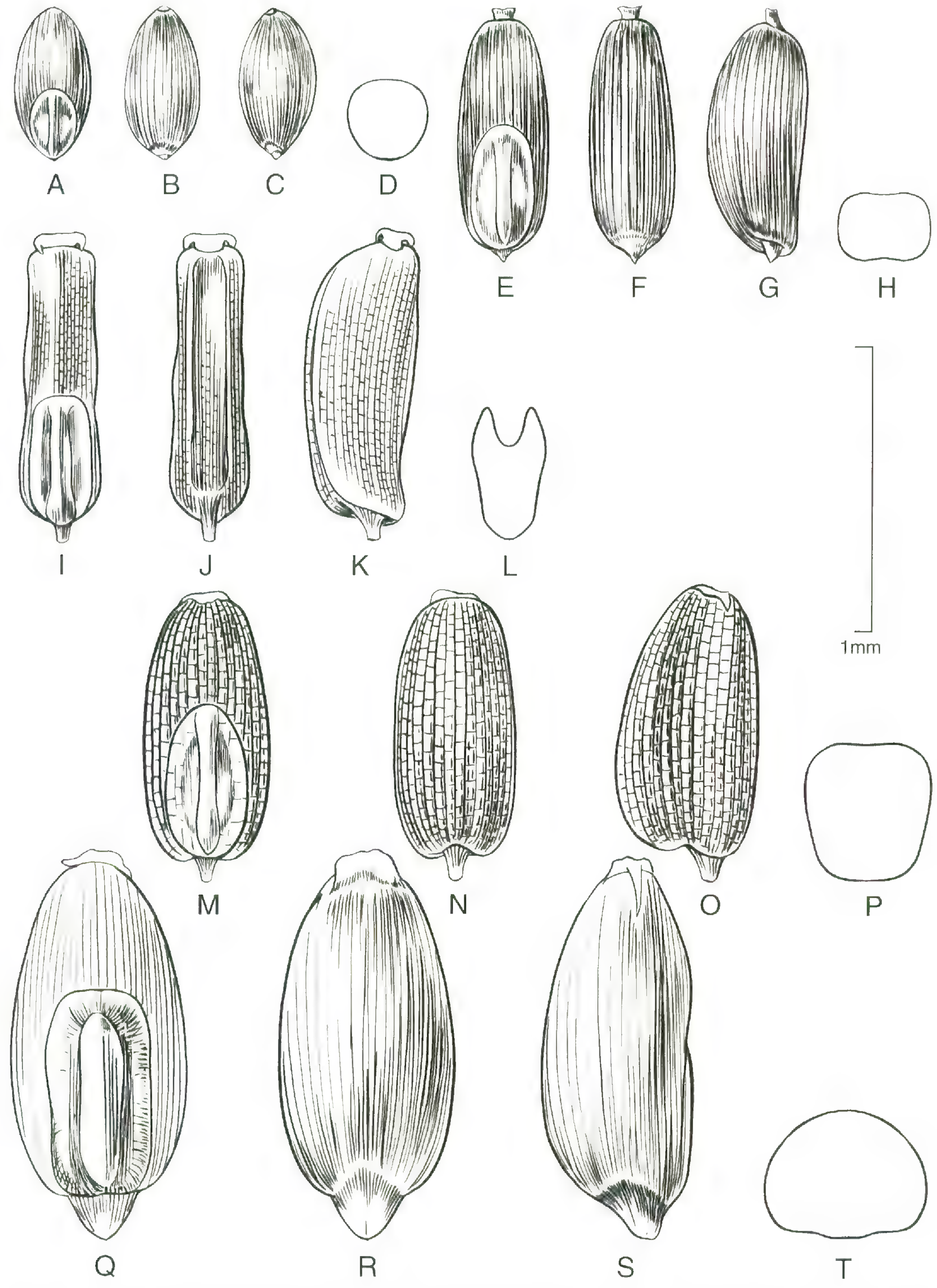

Figure 6. Caryopses of *Eragrostis* species. —A–D. *E. tenella* (*S. Llatas Q. 1180*). —E–H. *E. pilosa* (*S. Llatos 1107*). —I–L. *E. tenuifolia* (*P. Nuñez V. & E. Bengoa 8731*). —M–P. *E. pectinacea* (*P. M. Peterson & N. F. Refulio-Rodriguez 13981*). Q–T. *E. curvula* (*J. R. Reeder & C. G. Reeder 7311*). Dorsal views (A, E, I, M, Q); ventral views (B, F, J, N, R); lateral views (C, G, K, O, S); cross sections (D, H, L, P, T).

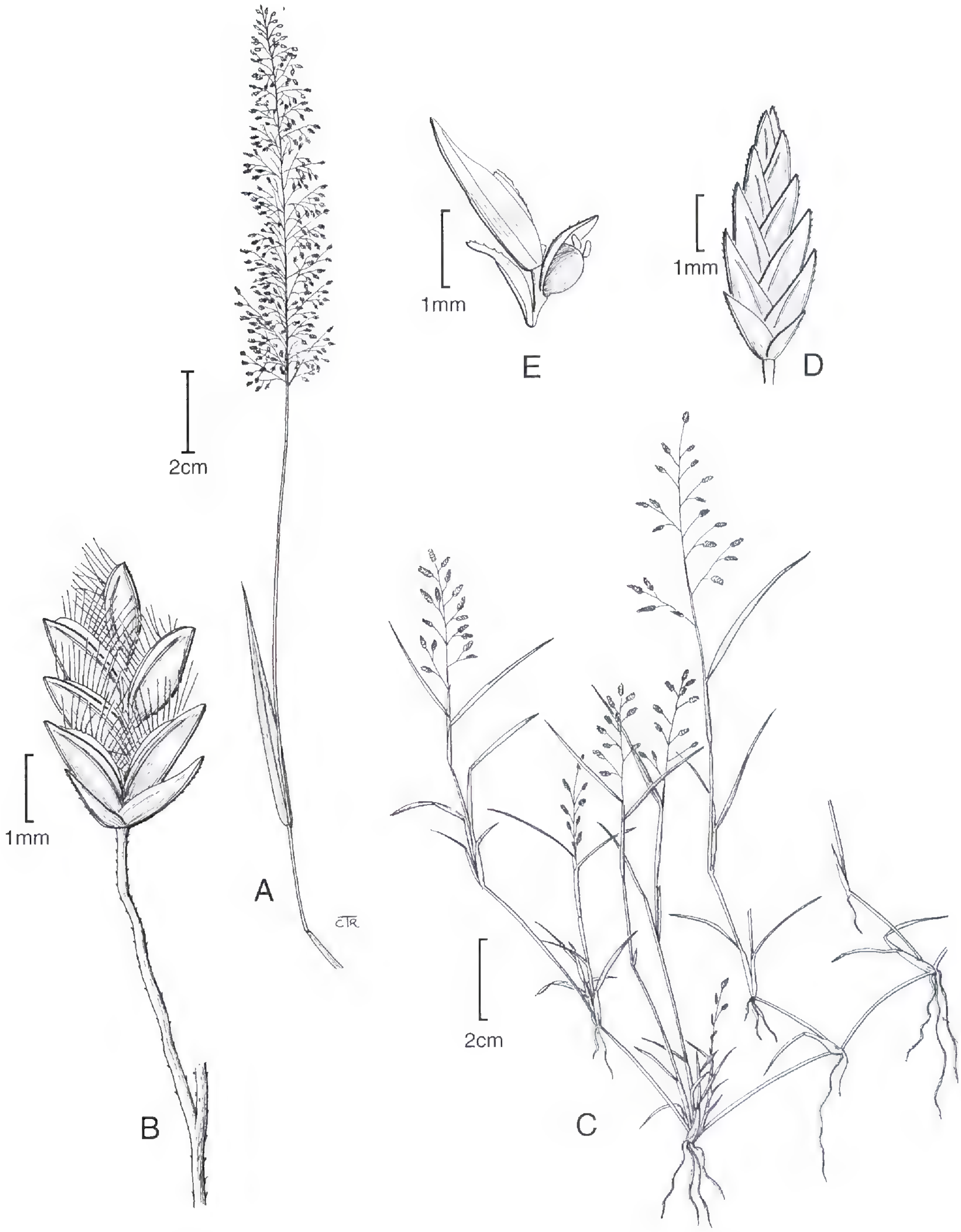

Figure 7. *Eragrostis tenella*. —A. Habit. —B. Spikelet. *Eragrostis hypnoides*. —C. Habit. —D. Spikelet. —E. Rachilla showing a palea and floret (left) and a palea and caryopsis (right).

small oblong glands located above the abaxial veins near the base, scaberulous above and pilose abaxially to glabrous, the papillose-based hairs up to 4 mm long. Panicles (6–)9–20 × 5–10 cm, oblong, open, primary branches 2–8 cm with glandular bands below the branch bases, 1 or 2 per node, the branches spreading 45°–90° from the rachises; secondary panicle branches composed of loosely overlapping spikelets; pulvini glabrous to sparingly ciliate; pedicels 1.8–5 mm, divaricate at maturity with a single glandular band near the middle, stout, scabrous. Spikelets 4–9 × 1.8–2.2 mm, 3- to 12-flowered, ovate-lanceolate, plumbeous to purplish; rachilla somewhat flattened with a few short,

scattered hairs mostly near the base; disarticulation acropetal, glumes first then lemmas, paleae persistent; glumes 1.2–2.4 mm, subequal ovate to lanceolate, membranous, usually purplish with hyaline margins; lower glume 1.2–2 mm, narrower than the upper; upper glume (1.8–)2–2.4 mm; lemmas 2–2.8 mm, ovate, membranous, plumbeous, lateral veins not evident, scaberulous along keel and near the apex; apex obtuse, occasionally mucronate, the mucro less than 0.2 mm; paleae 1.2–2.4 mm, shorter than the lemma, membranous, scaberulous; apex obtuse to truncate; stamens 3, anthers 0.4–0.6 mm, yellowish. Caryopses ca. 0.5–1 mm, rectangular-prismatic, striate and reticulate, rectangular with nearly equal sides in cross section, without a readily apparent ventral groove or with a shallow ventral groove.

Distribution and habitat. Native to northwestern Argentina, this is the first report in Peru from Departamentos Huánuco and Lima (Nicora, 1998); loose shale outcrops and gravelly river flats; 2100–2500 m.

Phenology. Flowering November through April.

Comments. This is the first record of this species for Peru, and, until now, *Eragrostis andicola* was known only from the province of Jujuy in northwestern Argentina. The Peruvian specimens are taller than specimens from Argentina where Nicora (1998) lists the culms as being 13–30 cm. Otherwise, important diagnostic characters of this species include the pedicels with a single glandular band near the middle, blades with scattered, small oblong glands located abaxially above the veins, and sheaths/blades with stiff, papillose-based hairs.

The specimen *R. E. Fries 924* at UPS has been selected as the lectotype because it is deposited at the institution in which the author worked and deposited types.

Specimens examined. ARGENTINA. **Jujuy:** Moreno, *R. E. Fries 924a* (syntype, BAA 998, CORD). PERU. **Huánuco:** *J. F. Macbride 3516* (F, US). **Lima:** Prov. Huarochirí, Matucana, *J. F. Macbride 2926* (F, US).

3. **Eragrostis atrovirens** (Desf.) Trin. ex Steud., Nom. Bot. (ed. 2) 1: 562. 1840. Basionym: *Poa atrovirens* Desf., Fl. Atlant. 1: 73, t. 14. 1798. TYPE: Algeria. E Barbaria, *Desfontaines 160* (holotype, FI not seen; isotypes, BAA 1006!, LE!, P!). Figures 4A–D; 9A, B.

Perennial, caespitose; culms (70–)75–130 cm tall, erect to geniculate at base. Leaf sheaths 1/3–2/3 as long as the internodes, glabrous, long ciliate at summit, the hairs up to 4 mm; ligule 0.1–0.3 mm, ciliate; blades (5–)8–20 × (0.1–)0.2–0.3(–0.4) cm, flat to involute, glabrous below and scaberulous above, long ciliate at base. Panicles (7–)10–20(–28) × (2.5–)4–15 cm, open, ovate, primary branches (3–)5–10(–13) cm, wiry, somewhat capillary, naked basally, branches diverging 20°–60° from the rachises; pulvini glabrous to sparingly ciliate, the hairs less than 2 mm; pedicels 1–10 mm, appressed, scaberulous. Spikelets 6–10(–19) × 1.4–2.4 mm, 10- to 22-flowered, ovate-lanceolate, plumbeous to purplish; disarticulation acropetal, glumes first, then the florets; glumes 1.2–1.7 mm, subequal, lanceolate to ovate, membranous; lower glume 1.2–1.4 mm, narrower than upper glume; upper glume 1.4–1.7 mm; lemmas (1.5–)1.7–2 mm, broadly ovate, leathery, lateral veins evident, scaberulous along keel; apex acute; paleae 1.4–1.9 mm, hyaline, scaberulous along keels; apex acute to obtuse; stamens 3, anthers 0.7–0.9 mm, reddish purple. Caryopses 0.6–0.9 mm, obovoid to ellipsoid, opaque, striate and minutely reticulate, circular in cross section, without a ventral groove, reddish brown. $2n = 20, 40, 60$ (Bir & Sahni, 1988).

Distribution and habitat. Native in northern Africa and Asia; introduced in U.S.A., Mexico, Central America, Caribbean, Bolivia, Chile, Argentina, Peru, and Venezuela (Nicora, 1998; Peterson & Boechat, 2001); railways, roads, beaches, and ditches, often in wet sandy soils of Amazonian region; 1–700 m.

Phenology. Flowering January through February.

Specimens examined. PERU. **Huánuco:** Prov. Leoncio Prado, Dist. Rupa Rupa, *I. Sánchez-Vega 9651* (CPUN, US). **Loreto:** Prov. Maynas, Iquitos, carretera to Santo Tomás, *M. Rimachi Y. 8470* (US).

4. **Eragrostis attenuata** Hitchc., Contr. U.S. Natl. Herb. 24(8): 340. 1927. TYPE: Peru. Arequipa: sandy slopes of hills ca. 3 km back of Mollendo, 17 Nov. 1923, *A. S. Hitchcock 22424* (holotype, US 1164909!; isotypes, BAA 1007!, US 1164361!). Figures 3A–D; 10A–C.

Sporobolus scaber Phil., Fl. Atacam. 54. 1860. TYPE: Chile. Cachinal de la Costa, 1900 m, *R. Philippi 118* (holotype, SGO-PHIL 118 not seen; isotypes, BAA 2928 [fragm.]!, SGO 45543 not seen, US 2891484 [fragm.] ex SGO-PHIL 118! & photo!).

Perennial, caespitose, forming innovations at the base; culms 15–40(–60) cm tall, stiffly, erect to widely spreading, geniculate below, glabrous, not branched at the upper nodes, innovations extravaginal formed below. Leaf sheaths nearly as long as the internodes above, ciliate at the summit with fine white hairs at the throat and along margins,

Figure 8. *Eragrostis andicola* (*J. F. Macbride 2926, 3516*). —A. Habit. —B. Sheath and blade. —C. Spikelet. *Eragrostis lurida* subsp. *contracta* (*P. M. Peterson & N. F. Refulio-Rodriguez 13917A*). —D. Panicle. —E. Spikelet.

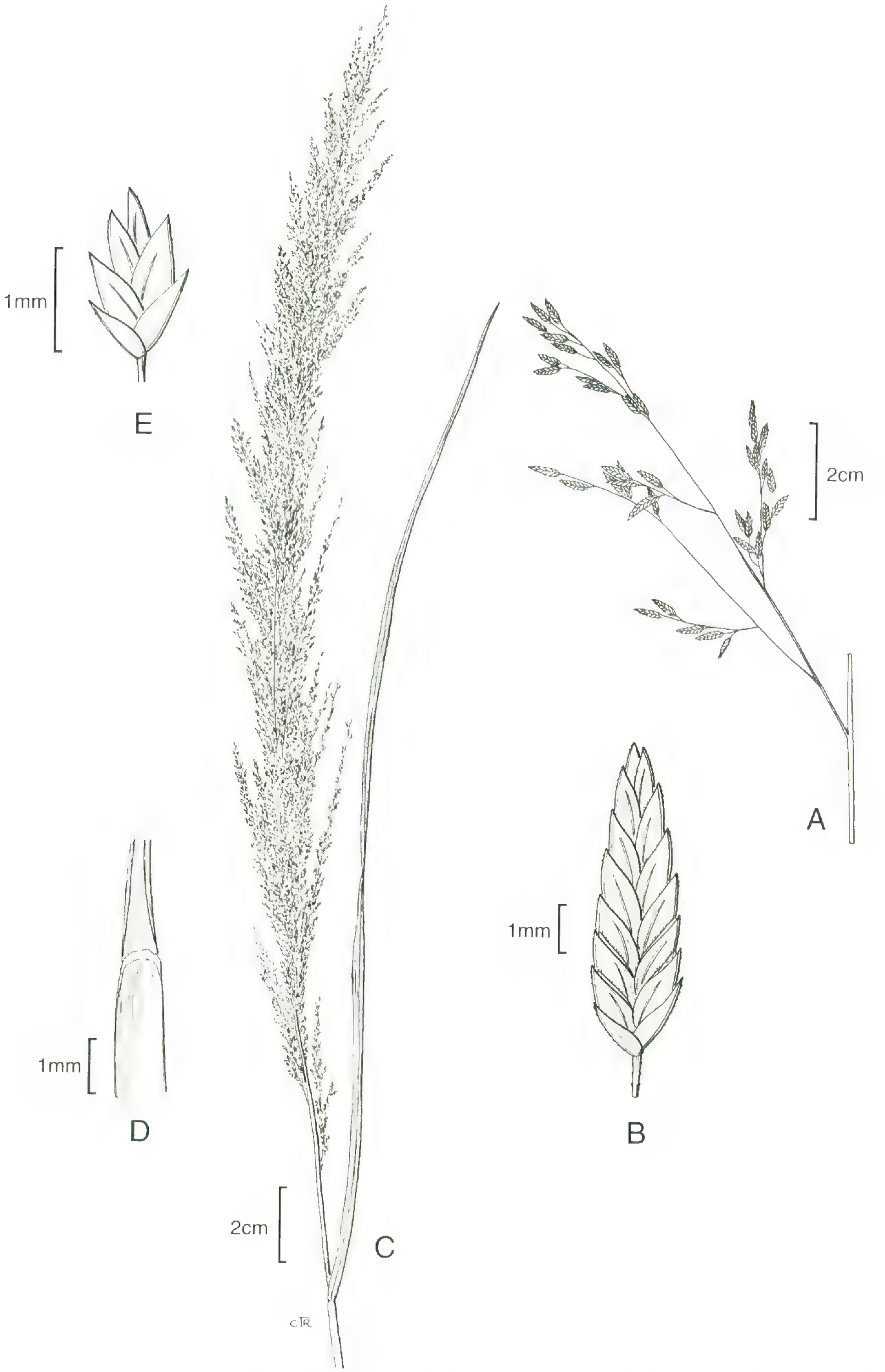

Figure 9. *Eragrostis atrovirens*. —A. Paniele branch. —B. Spikelet. *Eragrostis japonica*. —C. Paniele and flag leaf. —D. Ligule. —E. Spikelet.

Figure 10. *Eragrostis attenuata* (*M. O. Dillon & D. Dillon 3879*). —A. Habit. —B. Panicle branch. —C. Spikelet. *Eragrostis nigricans* (*P. M. Peterson & N. F. Refulio-Rodriguez 13855*). —D. Habit. —E. Panicle branch. —F. Spikelet.

sometimes with similar hairs on the collar; ligule ca. 1 mm, ciliate; blades 3–7 × 0.2–0.3 cm, flat or involute when dry, short pilose, the hairs 0.4–1.5 mm, shiny, whitish, and minutely tuberculate at base. Panicles 10–25(–40) × 0.2–0.5 cm, closed, densely flowered, cylindrical and narrowly spicate, interrupted below, rachis glabrous, primary branches 1–1.5(–4) cm, ascending and tightly appressed, solitary, flowered to the base; pedicels 1–1.5 mm, short, appressed. Spikelets 2.5–4 × 0.9–1.1 mm, (1 to)3- to 4(to 6)-flowered, oblong to oval, dark reddish purple, florets imbricate; disarticulation between the florets with rachilla joint attached; glumes 0.8–1.4 mm, unequal, hyaline, lanceolate to acuminate, slightly keeled; lower glume 0.8–1 mm; upper glume 1–1.4 mm, 1-nerved, scaberulous along the keel; lemmas 1.2–1.8 mm, ovate to ovate-lanceolate, scabrous throughout, keeled, lateral nerves conspicuous, scaberulous; apex obtuse; paleae 1.1–1.3 mm, nearly as long as lemma, keels minutely scaberulous; apex acute to obtuse; stamens 3, anthers ca. 0.3 mm. Caryopses 0.6–0.8 mm, ellipsoid, striate, laterally flattened, elliptical in cross section, reddish brown, smooth, not grooved ventrally.

Distribution and habitat. Endemic along the coast of southern Peru south to northern Chile (Nicora, 1998; Peterson & Boechat, 2001); occurs in lomas vegetation; 5–1000 m.

Phenology. Flowering September through November.

Specimens examined. PERU. **Arequipa:** Prov. Arequipa, Pampas de la Joya, *C. Vargas C. 017943* (CUZ); Prov. Camaná, ca. 32 km SE of Camaná, *M. O. Dillon & D. Dillon 3879* (F, US); Prov. de Caravelí, lomas de Arequipa, *R. Ferreyra 6478* (US, USM); Prov. Islay, near the Puerto Mollendo, *I. M. Johnston 3538* (US). **Ica:** Prov. Nasca, Lomas de Marcona, *R. Ferreyra 13371* (US, USM).

5. Eragrostis cilianensis (All.) Vignolo ex Janch., Mitt. Naturwiss. Vereins Univ. Wien, n.s., 5: 110. 1907. Basionym: *Poa cilianensis* All., Fl. Pedem. 2: 246. 1785. *Eragrostis megastachya* var. *cilianensis* (All.) Asch. & Graebn., Syn. Mitteleur. Fl. 2: 371. 1900. *Eragrostis cilianensis* (All.) F. T. Hubb., Philipp. J. Sci. 8(3): 159–161. 1913. *Erosion cilianense* (All.) Lunell, Amer. Midl. Naturalist 4: 221. 1937. *Eragrostis multiflora* var. *cilianensis* (All.) Maire, Bull. Soc. Hist. Nat. Afrique N. 30: 369. 1939. TYPE: Italy. Ciliani, *Bellardi s.n.* (lectotype, designated by Vignolo, Malpighia 18: 380. 1904, TO 8242 not seen; isotypes, BRI not seen, K photo neg. 19571!). Figures 2A–D; 11A–C.

Annual, caespitose; culms 15–45(–65) cm tall, erect or decumbent and prostrate, glabrous and shiny, sometimes with crateriform glands below the nodes. Leaf sheaths 2/3 the length of the internodes above, occasionally longer, glabrous, occasionally glandular, densely ciliate at the summit, the hairs often elongate up to 5 mm; ligules 0.4–0.8 mm, ciliate; blades (1–)5–20 × (0.1–)0.3–0.5(–1) cm, flat to loosely involute, mostly glabrous below, scaberulous above occasionally with widely spaced elongate hairs, sometimes glandular near margins. Panicles (3–)5–16(–20) × 2–8.5 cm, condensed to open, oblong to ovate, primary branches 0.4–5 cm, ascending, appressed or diverging 20 –80 from the rachises; pulvini glabrous or ciliate; pedicels 0.2–3 mm, erect, spreading to appressed. Spikelets 6–20 × 2–4 mm, 10- to 40-flowered, ovate-lanceolate, florets imbricate, plumbeous to greenish or hyaline; disarticulation acropetal, between the florets from the base upward, usually the entire floret, rachillas persistent; glumes 1.2–2.6 mm, subequal, broadly ovate to lanceolate, subhyaline, membranous, keeled, usually with crateriform glands along the keel, 1- or 3-nerved, scaberulous toward apex of keel; lower glume 1.2–2 mm, usually 1-nerved; upper glume 1.2–2.6 mm, often 3-nerved; lemmas 2–2.8 mm, broadly ovate, membranous, lateral nerves evident, strongly keeled, keels with 1 to 3 crateriform glands; apex obtuse to acute; paleae 1.2–2.1 mm, hyaline, keels scaberulous, sometimes ciliate, the cilia less than 0.3 mm; apex obtuse to acute; stamens 3, anthers 0.2–0.5 mm, yellow. Caryopses 0.5–0.7 mm, globose to broadly short ellipsoid, striate and reticulate, circular to elliptical in cross section, reddish brown. $2n = 20, 40$ (Bir & Sahni, 1988).

Distribution and habitat. Native to Europe; introduced in southern Canada, U.S.A., Mexico, Central America, Caribbean, Argentina, Bolivia, Brazil, Colombia, Ecuador, Paraguay, Peru, Uruguay, and Venezuela (Laegaard & Peterson, 2001; Peterson & Boechat, 2001); a weed near cultivated fields, pastures, roadsides, and disturbed habitats; 0–3000 m.

Phenology. Flowering March through July.

Comments. Specimens from the provinces of Azuay and Loja, Ecuador, have elongate hairs on the adaxial surface of the blades and lack crateriform glands on the spikelets, whereas specimens from other provinces in Ecuador and throughout South America lack the elongate hairs on the blades and generally have crateriform glands on the spikelets (Laegaard & Peterson, 2001).

Figure 11. *Eragrostis cilianensis*. —A. Habit. —B. Spikelet. —C. Rachilla showing palea (left) and floret (right). *Eragrostis ciliaris*. —D. Habit. —E. Spikelet. —F. Palea.

Specimens examined. PERU. **Arequipa:** Prov. Castilla, Paccai-chacra, *C. Vargas C. 019367* (CUZ). **Cajamarca:** Prov. Cajamarca, 40 km N of Cajabamba & 13 km S of Ichocan, *P. M. Peterson & N. F. Refulio-Rodriguez 14004* (US, USM); Prov. Contumazá, entre Chilete y Tembladera, *I. Sánchez Vega & W. Ruiz V. 665* (CPUN); Prov. Jaén, 1 km N of Chamayo on Hwy. 5N toward Jaén, *P. M. Peterson & N. F. Refulio-Rodriguez 15045* (US, USM). **Ica:** Prov. Nasca, 15 km NW of Nasca along Hwy. 1, *J. T. Columbus, J. M. Porter & E. H. Roalson 3519* (RSA, US). **La Libertad:** Prov. Pacasmayo, entre Paiján y San Pedro, *D. N. Smith 4226* (US). **Lambayeque:** Prov. Chiclayo, Tucumán–Chongoyape, *A. Sagástegui A. et al. 11468* (US); Prov. Lambayeque, betw. Lambayeque & Motupe, *J. T. Columbus, J. M. Porter & E. H. Roalson 3439* (RSA, US). **Lima:** Prov. Chancay, al E del valle de Sayán, *T. H. Goodspeed 33034*

(US). **Moquegua:** Prov. Moquegua, entre Moquegua y Torata, *I. Weberbauer 7138* (US). **Piura:** Prov. Huancabamba, 10 km N of Sondor & 3 km S of Huancabamba, *P. M. Peterson & N. F. Refulio-Rodriguez 15169* (US, USM); Pabur, Buenos Aires, *R. Ferreyra 6017* (US, USM); 15 km entre Piura y Sullana, E de la carretera Panamericana, *I. Sánchez Vega & J. Guevara B. 6188* (CPUN, HAO). **Tumbes:** Caleta Santa Cruz, entre Tumbes y Zorritos, *R. Ferreyra 10693* (US, USM).

6. Eragrostis ciliaris (L.) R. Br. var. **ciliaris**, Narr. Exped. Zaire 478. 1818. Basionym: *Poa ciliaris* L., Syst. Nat. (ed. 10) 875. 1759. *Megastachya ciliaris* (L.) P. Beauv., Ess. Agrostogr. 74, 167, 174. 1812. *Cynodon ciliaris* (L.) Raspail, Ann. Sci. Nat., Bot. 5: 302. 1825. *Eragrostis ciliaris* (L.) Nees, Fl. Bras. Enum. Pl. 2: 512–514. 1829. TYPE. Jamaica. *Browne s.n.* (lectotype, designated by Hitchcock, Contr. U.S. Natl. Herb. 12: 121. 1908. LINN 87.66!). Figures 2E–H; 11D–F.

Annual, caespitose; culms (3–)9–75 cm tall, erect or geniculate in the lower portion, not rooting at the lower nodes, glabrous. Leaf sheaths 1/2–3/4 as long as the internodes, hairy on the margins and at the apices, hairs to 4 mm; ligules 0.2–0.5 mm; blades 1.8–12(–15) × 0.2–0.5 cm, usually flat, occasionally involute, glabrous or ciliate basally. Panicles 1.7–17 × 0.2–1.5 cm, cylindrical, contracted, spikelike, branches forming glomerate lobes or sometimes more open, often interrupted in the lower portion; primary branches 0.4–4 cm, ascending, tightly appressed; pulvini usually glabrous, occasionally sparsely pilose; pedicels 0.1–1 mm, erect, shorter than the spikelets, glabrous. Spikelets 1.8–3.2 × 1–2 mm, 6- to 11-flowered, elliptical-ovate to ovate-lanceolate, yellowish brown, sometimes with a purple tinge, densely packed next to one another forming glomerate lobes; disarticulation basipetal, glumes persistent; glumes 0.7–1.6 mm, subequal, ovate to lanceolate, keels scabridulous, veins commonly green, apices acute; lower glume 0.7–1.2 mm; upper glume 1–1.6 mm; lemmas 0.8–1.3 mm, elliptical-ovate to lanceolate, membranous, keels scabridulous, lateral veins evident; apex obtuse to acute; paleae 0.8–1.3 mm, membranous, keels prominently ciliate, cilia 0.2–0.8 mm, stiff and pectinate-thickened near base; apex obtuse to acute; stamens 2, anthers 0.1–0.3 mm, purplish. Caryopses 0.4–0.5 mm, ovoid, striate, slightly dorsally flattened, elliptical in cross section, reddish brown. $2n = 20, 40$ (Bir & Sahni, 1988).

Distribution and habitat. Apparently native to the Paleotropics; introduced and naturalized in Mexico, U.S.A., Central America, Caribbean, Argentina, Bolivia, Colombia, Ecuador, Guianas, Paraguay, Peru, and Venezuela (Nicora, 1998; Peterson & Boechat, 2001); growing along roadsides, on waste sites, in xerothermic vegetation, and sometimes in saline habitats; 0–1400 m.

Phenology. Flowering October through July.

Specimens examined. PERU. **Cajamarca:** Prov. Cajamarca, entre Cajamarca y Cumbe Mayo, W of Cajamarca, *I. Sánchez Vega 4125* (CPUN); Prov. Jaén, 6 km W of Hwy. 5N up Río Tabaconas toward Tamborillo, *P. M. Peterson & N. F. Refulio-Rodriguez 15085* (US, USM); 1 km N of Chamayo on Hwy. 5N toward Jaén, *P. M. Peterson & N. F. Refulio-Rodriguez 15046* (US, USM); Prov. San Ignacio, confluencia del Río Tamboraza–Chinchipe, *E. Anderson 1057* (US). **Cusco:** Prov. Convención, Echarati, *C. Vargas C. 21288* (CUZ). **Huánuco:** Prov. Leoncio Prado, Tingo María, *H. A. Hallar 20709* (US). **Junín:** Prov. Chanchamayo, San Ramón, *O. Velarde 2840* (US). **La Libertad:** Prov. Trujillo, Mócan, Hacienda Casagrande, *A. Sagástegui A. 3976* (US). **Lambayeque:** Prov. Lambayeque, betw. Chochope & La Ramada, *J. T. Columbus, J. M. Porter & E. H. Roalson 3444* (RSA, US); El Remanzo, Olmos, 2 Nov. 1991, *S. Llantos Q. s.n.* (HAO). **Loreto:** Prov. Maynas, Playa Timicurillo, *S. McDaniel & M. Rimachi 23073* (AMAZ); Vecindades de Iquitos, Mishuyacu, *E. Anderson 848* (US). **Piura:** Prov. Talara, entre Sullana y Talara, *A. Sagástegui A. 10927* (US). **Tumbes:** Prov. Tumbes, La Esperanza, near Tumbes, *A. Sagástegui A. 3063* (US). **Ucayali:** Prov. Contamana, ca. a la carretera del Oriente, *S. McDaniel 14049* (AMAZ).

7. Eragrostis curvula (Schrad.) Nees, Fl. Afr. Austral. Ill. 397. 1841. TYPE: South Africa. Cape Province: Cape of Good Hope, *Hesse s.n.* (holotype, LE!; isotype, LE-TRIN 2327.01 [lower middle specimen]!). Figures 6Q–T; 12A–C.

Perennial, caespitose, forming innovations at the base; culms (45–)60–150 cm tall, erect, glabrous or glandular. Leaf sheaths 1/3–2/3 the length of the internodes, with scattered hairs, hairs to 9 mm; ligules 0.6–1.3 mm, ciliate; blades 12–50(–65) × 0.1–0.3 cm, flat to involute, abaxial surfaces glabrous, sometimes scaberulous, adaxial surfaces with scattered hairs basally, hairs to 7 mm. Panicles 16–35 (–40) × (4–)8–24 cm, ovate to oblong, open; primary branches 3–14 cm, diverging 10°–80° from the rachises; pulvini glabrous or not, the hairs up to 3 mm; pedicels 0.5–5 mm, appressed, flexible. Spikelets 4–8.2(–10) × 1.2–2 mm, 3- to 10-flowered, linear-lanceolate, plumbeous to yellowish; disarticulation irregular to acropetal, proximal rachilla segments persistent; glumes 1.2–3 mm, subequal, lanceolate, hyaline; lower glume 1.2–2.6 mm; upper glumes 2–3 mm; lemmas 2–3.2 mm, ovate, membranous, lateral veins conspicuous; apex acute; paleae 2–3.2 mm, hyaline to membranous; apex obtuse; sta-

Figure 12. *Eragrostis curvula*. —A. Panicle. —B. Spikelet. —C. Floret. *Eragrostis lugens*. —D. Culm base. —E. Panicle. —F. Spikelet. —G. Rachilla showing palea (left) and lemma (right).

mens 3, anthers 0.8–1.3 mm, reddish brown. Caryopses 1–1.7 mm, ellipsoid to obovoid, strongly dorsally flattened, ventral surface with a shallow, broad groove or ungrooved, smooth to striate, mostly translucent, elliptical in cross section, light brown, bases often greenish. $2n = 40, 50, 60, 80$ (de Wet, 1954; Spies & Jonker, 1987).

Distribution and habitat. Native to southern Africa; introduced in the U.S.A., Mexico, Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Peru, Uruguay, and Venezuela (Nicora, 1998; Peterson & Boechat, 2001); rocky slopes, margins of woods, roadsides, waste ground, and often used in reclamation; 10–2900 m.

Phenology. Flowering in June.

Specimens examined. PERU. **Cajamarca:** Prov. Cajamarca, entre Cajamarca y Cumbe Mayo, W of Cajamarca, *I. Sánchez Vega 4125* (CPUN).

8. Eragrostis hypnoides (Lam.) Britton, Sterns & Poggenb., Prelim. Cat. 69. 1888. Basionym: *Poa hypnoides* Lam., Tabl. Encycl. 1: 185. 1791. *Megastachya hypnoides* (Lam.) P. Beauv., Ess. Agrostogr. 74, 167, 175. 1812. *Neeragrostis hypnoides* (Lam.) Bush, Trans. Acad. Sci. St. Louis 13: 180. 1903. *Erosion hypnoides* (Lam.) Lunell, Amer. Midl. Naturalist 4: 221. 1915. TYPE: Tropical America, *D. Richard s.n.* (holotype, P-LA!; isotypes, BAA 1041!, NY [fragm.] ex P, US 2850742 [fragm.] ex P!). Figures 2I–L; 7C–E.

Annuals, stoloniferous, mat-forming, without innovations; culms decumbent and rooting at the lower nodes, erect portion (2–)5–12(–20) cm tall, often branched, glabrous or hairy on the lower internodes. Leaf sheaths 1/3–1/2 as long as the internodes above, pilose on the margins, collars, and at the apices, hairs 0.1–0.6 mm; ligules 0.3–0.6 mm, ciliate; blades 0.5–2.5 × 0.1–0.2 cm, flat to involute, abaxial surfaces glabrous, adaxial surfaces appressed pubescent, hairs ca. 0.2 mm. Panicles 1–3.5 × 0.7–2.5 cm, terminal and axillary, ovate, open to somewhat congested; primary branches 0.1–0.5 cm, appressed to strongly divergent, glabrous; pulvini sparsely pilose or glabrous; pedicels 0.2–1 mm, ciliate. Spikelets 4–13 × 1–1.5 mm, 12- to 35-flowered, linear-oblong, often arcuate, loosely imbricate, greenish yellow to purplish; disarticulation acropetal, paleae persistent; glumes 0.4–1.2 mm, subequal, linear-lanceolate to lanceolate, hyaline; lower glume 0.4–0.7 mm; upper glume 0.8–1.2 mm; lemmas 1.4–2 mm, ovate, strongly 3-veined, veins greenish; apex acuminate; paleae 0.7–1.2 mm, hyaline, keels scaberulous; apex acute to obtuse; stamens 2, anthers 0.2–0.3 mm, brownish. Caryopses 0.3–0.5 mm, ellipsoid, faintly striate and reticulate, laterally flattened, somewhat translucent, elliptical in cross section, light brown. $2n = 20$ (Davidse & Pohl, 1972).

Distribution and habitat. Native to the Americas and found in North, Central, and South America (excluding Chile and Colombia) and the Caribbean (Nicora, 1998; Peterson & Boechat, 2001); found along muddy or sandy shores of lakes and rivers and moist disturbed sites; 0–500 m.

Phenology. Flowering August through February.

Specimens examined. PERU. **Cajamarca:** Prov. San Ignacio, al N de San Ignacio, *I. Sánchez Vega 3965* (CPUN). **Loreto:** Prov. Maynas, Santa María near Yurimaguas, *R. Ferreyra 5023* (US, USM); Iquitos, *F. Ayala 00676* (AMAZ). **Tumbes:** Prov. Tumbes, margen del Río Tumbes, *A. López M. & A. Sagástegui A. 4023* (US). **Ucayali:** Prov. Coronel Portillo, *A. Sagástegui A. & A. Aldave 5720* (US).

9. Eragrostis japonica (Thunb.) Trin., Mém. Acad. Imp. Sci. St.-Pétersbourg, Sér. 6, Sci. Math. 1(4): 405. 1830. Basionym: *Poa japonica* Thunb., Fl. Jap. 51. 1784. *Eragrostis tenella* var. *japonica* (Thunb.) Roem. & Schult., Syst. Veg. 2: 576. 1817. *Diandrochloa japonica* (Thunb.) A. N. Henry, Bull. Bot. Surv. India 9: 290. 1968. *Roshevitzia japonica* (Thunb.) Tzvelev, Novosti Sist, Vyss. Rast. 7: 50. 1970[1971]. TYPE: Japan, *Herb. Thundberg 2252* (holotype, UPS not seen, microfiche IDC 1036!, K photo!; isotype, BRI fragm. not seen). Figures 2M–P; 9C–E.

Poa glomerata Walter, Fl. Carol. 80. 1788. *Eragrostis glomerata* (Walter) L. H. Dewey, Contr. U.S. Natl. Herb. 2(3): 543. 1894. *Megastachya glomerata* (Walter) Schult., Mant. 2: 327. 1824. *Diandrochloa glomerata* (Walter) Burkart, Bol. Soc. Argent. Bot. 12: 287. 1968. TYPE: U.S.A. South Carolina: *Walter s.n.* (holotype, BM!).

Annual, caespitose; culms 25–100(–115) cm tall, erect, sometimes geniculate below, branching from the lower and middle nodes, glabrous and somewhat shiny below the nodes. Leaf sheaths 3/4–7/8 as long as the internodes above, glabrous at the summit and along the upper margins; ligules 0.4–0.6 mm, a delicate membrane, glabrous; blades (4–)15–20(–25) × 0.15–0.6 cm, flat, glabrous below and scaberulous above, sometimes auriculate near the base. Panicles 15–40 × 0.8–5 cm, lanceoloid, contracted, interrupted below, the ascending, often appressed primary branches 2–10 cm, spreading up to 30° from the rachises, the branches scaberulous and shiny, fliferous near base; pulvini glabrous; pedicels 0.5–1.5 mm, erect and sinuous. Spikelets 2.2–3.8 × 0.8–1.3 mm, 4- to 12-flowered, oblong to narrowly lanceolate, yellowish brown to whitish and hyaline; disarticulation basipetal, rachillas and glumes persistent; glumes 0.6–1 mm, subequal, ovate to ovate-lanceolate, hyaline, faintly keeled, scaberulous along the keel; upper glume without a midnerve; lemmas 0.9–1.2 mm, ovate, hyaline, lateral nerves conspicuous below, greenish, keeled, glabrous along the keel; apex acute; paleae 0.6–0.8 mm, hyaline, scaberulous along the keel near the apex; apex acute, often bifid; stamens 2, anthers 0.1–0.2 mm, whitish to light brown. Caryopses 0.3–0.4 mm, obovoid, smooth or minutely irregularly striate, slightly dorsally flattened, pericarp often loose, elliptical in cross section, reddish brown. $2n = 20, 60$ (Pohl & Davidse, 1971

reported for *E. glomerata*; Christopher & Abraham, 1974).

Distribution and habitat. Native in tropical regions of Asia; introduced in U.S.A., Mexico, Central America, Caribbean, Argentina, Bolivia, Brazil, Ecuador, Guyana, Paraguay, Peru, Uruguay, and Venezuela (Nicora, 1998; Peterson & Boechat, 2001); occurs in moist areas along rivers and streams, usually in sandy soils; 0–400 m.

Phenology. Flowering January through November.

Specimens examined. PERU. **Cajamarca:** Prov. Jaén, 1 km N of Chamayo on Hwy. 5N toward Jaén, *P. M. Peterson & N. F. Refulio-Rodriguez 15053* (US, USM). **Huánuco:** Prov. Pachitea, Bosque Nacional de Iparia, *J. Schunque V. 2094* (US). **La Libertad:** Prov. Trujillo, Mócan, Hacienda Casagrande, *A. Sagástegui A. 3977* (US). **Lambayeque:** Prov. Reque, Río Reque, *S. Llatas Q. 193* (US). **Loreto:** Iquitos, *E. Asplund 14802* (US). **Madre de Dios:** Parque Nacional del Manu, Cocha–Cashu, Río Manu, *R. Foster 9769* (US). **Piura:** Hacienda Buenos Aires, campos de arroz, *E. Anderson 569* (US).

10. Eragrostis lugens Nees, Fl. Bras. Enum. Pl. 2: 505–506. 1829. *Eragrostis pilosa* var. *lugens* (Nees) Griseb., Abh. Königl. Ges. Wiss. Göttingen 24: 290. 1879. TYPE: Brazil. Meridionalis, *F. Sellow s.n.* (lectotype, designated by Davidse, Fl. Mesoamericana 6: 271. 1994, US 732957!; isotypes, B not seen, BAA 2932!). Figures 11–L; 12D–G.

Perennial, caespitose, forming innovations at base; culms 30–70 cm tall, erect, sometimes geniculate below, glabrous and somewhat shiny below the nodes. Leaf sheaths 1/2–2/3 as long as the internodes above, ciliate at the summit and along the upper margins; ligules 0.2–0.3 mm, ciliate; blades (5–)8–22 × 0.1–0.2(–0.22) cm, involute, rarely flat, mostly glabrous above and below, scaberulous toward the apex and along margins, sometimes with scattered hairs along the margins, the hairs up to 7 mm. Panicles 16–28 × 10–21 cm, open, ovate, the ascending primary branches 0.6–15 cm, spreading up to 100° from the rachises, the branches scaberulous, not floriferous near base; secondary branches composed of loosely overlapping spikelets; pulvini ciliate, the hairs up to 7 mm long; pedicels 1.4–5(–7) mm, erect, wiry, spreading. Spikelets 2–4.5(–5) × 0.5–1.1 mm, 2- to 7-flowered, narrow lanceolate, light plumbeous to reddish purple; disarticulation acropetal, paleae and rachilla persistent; glumes 0.6–1.4 mm, subequal, broadly ovate to narrowly lanceolate, hyaline, keeled, scaberulous along the keel, sometimes reddish purple; lower glume 0.6–1 mm; upper glume 1.1–1.4 mm, usually broader than the lower; lemmas 1.2–1.8 mm, broadly ovate, membranous, distal margins hyaline, lateral nerves obscure to barely evident, keeled, scaberulous along the keel near apex; apex acute; paleae 1.1–1.7 mm, membranous to partially hyaline, scaberulous along keels; apex obtuse; stamens 3, anthers 0.2–0.7 mm, reddish purple. Caryopses 0.5–0.6 mm, obovoid to somewhat prism-shaped, terete to somewhat laterally flattened, with a weak ventral groove, striate and reticulate, usually opaque, irregularly obovate to triangular in cross section, faintly reddish brown to whitish. $2n = 40, 80$ (Gould, 1958).

Distribution and habitat. Native to the Americas with a broad distribution from U.S.A., Mexico, Central America, Argentina, Bolivia, Brazil, Colombia, Ecuador, Peru, Uruguay, and Venezuela (Nicora, 1998; Peterson & Boechat, 2001); sandy dunes, river banks, near cultivated fields, and open slopes; 0–2200 m.

Phenology. Flowering in October.

Comments. The specimen *F. Sellow s.n.* was selected as lectotype by Davidse (1994), and his lectotypification was accepted by Boechat and Longi-Wagner (2001).

Specimens examined. PERU. **Apurímac:** Prov. Abancay, Tablachaca, *C. Vargas C. 015695* (CUZ).

11. Eragrostis lurida J. Presl, Reliq. Haenk. 1(4–5): 276. 1830. *Poa lurida* (J. Presl) Kunth, Enum. Pl. 1: 342. 1833. TYPE: Peru. *T. Haenke s.n.* (holotype, PR not seen; isotypes, MO 2111146!, PR not seen, US 2941523 [fragm.] ex PR!).

Perennial, caespitose, with innovations at base; culms (5–)15–75 cm tall, erect, sometimes slightly geniculate below, glabrous or occasionally with a tuft of hairs below the nodes, the hairs less than 1 mm, sometimes with an occasional elliptical orange gland. Leaf sheaths 3/4 to about as long as the internodes above, densely white ciliate at the summit and along the margins, sometimes with scattered hairs on the abaxial surface; ligules 0.5–0.9 mm, ciliate; blades 1.5–16.5 × 0.15–0.35(–0.5) cm, flat to involute, glabrous and sometimes shiny below and scaberulous above. Panicles 3–35 × 0.5–5(–7) cm, narrowly ovate to spicate, contracted and condensed into tightly glomerate lobes, interrupted near base, spicate to narrowly ovate, rachis glabrous, the ascending primary branches 0.6–5 cm, densely flowered, appressed or spreading 20°–80° from the rachises, floriferous near base; secondary branches condensed into tightly glomerate lobes of spikelets; pulvini ciliate or glabrous; pedicels 0.1–1 mm, ascending and appressed, wiry, scabrous. Spikelets 2.5–6 × 1–2.1 mm, 3- to 10-flowered, narrowly lanceolate to ovate, inflated to slightly compressed, dark to light

plumbeous, sometimes purple-tinged, rachilla often ciliate; disarticulation acropetal with glumes first, then lemmas falling individually, paleae persistent on rachilla; glumes 1–2 mm, subequal, broadly ovate to lanceolate, membranous, margins hyaline, keeled, scaberulous along keel, sometimes 3-nerved; lower glume 1–1.5 mm; upper glume 1.2–2 mm; lemmas 1.6–2.4 mm, broadly ovate, membranous, lateral nerves conspicuous, evident, keeled; apex acute, scaberulous, the minute prickle hairs appearing as whitish dots under 10–20×; paleae 1.5–2.3 mm, membranous to partially hyaline, scaberulous along the keels; apex truncate to obtuse; stamens 3, anthers 0.4–0.6 mm, reddish purple. Caryopses 0.6–0.8 mm, obovoid to ellipsoid, striate and reticulate, sometimes with a weak ventral groove, rectangular with the lateral sides angled in cross section, light reddish brown.

Distribution and habitat. Native to the central Andes from Bolivia, Colombia, Ecuador, and Peru (Nicora, 1998; Laegaard & Peterson, 2001); dry rocky hillsides, slopes, sandy roadsides, and rocky alluvial fans; 2000–3800 m.

Phenology. Flowering December (one collected in September) through July.

Key to the Subspecies of *Eragrostis lurida*

11a. Primary branches spreading; panicles 2–5(–7) cm wide, narrowly ovate . . . . . *E. lurida* subsp. *lurida*

11b. Primary branches appressed and overlapping on the rachis; panicles 0.5–2 cm wide, spicate . . . . . *E. lurida* subsp. *contracta*

11a. Eragrostis lurida J. Presl subsp. **lurida**. Figures 1A–D; 13A–Q.

Eragrostis contristata Nees & Meyen, Gramineae 31–32. 1841. TYPE: Peru. Laguna de Titicaca, Apr. 1831, *Meyen s.n.* (holotype, B not seen; isotypes, BAA-1021 [fragm.] ex B!, LE-TRIN 2326.01!, P!, US 2891461 [fragm.]!).

Eragrostis bahiensis var. *boliviensis* Henrard, Meded. Rijks-Herb. 40: 68. 1921. TYPE: Bolivia. La Paz: Larecaja, Sorata, 2900 m, Feb. 1858, *G. Mandon 1332* (holotype, L 908,88-303 not seen; isotypes, K!, L 908,88-342 not seen, P!, US 3161127!, US 256470!, US 911775 [fragm.] ex L!).

Panicles 2–5(–7) cm, narrowly ovate, primary branches spreading 20°–80° from the rachises.

Specimens examined. PERU. **Ancash:** Prov. Bolognesi, 8 km E of Raquia & 2 km W of Cajacay on Ruta 02-104, *P. M. Peterson, N. F. Refulio-Rodriguez, A. Cano, M. LaTorre & I. Salinas 17881* (US, USM); Prov. Huari, 21 km S of Huari on rd. toward San Marcos at Río Mosna crossing, *P. M. Peterson & N. F. Refulio-Rodriguez 13856* (US, USM). **Apurímac:** Prov. Abancay, Tablachaca, *C. Vargas C. 015701* (CUZ, USM); Prov. Andahuaylas, near Huancarama, *C. Vargas C. 008836* (CUZ). **Ayacucho:** Prov. Huamanga, Cerro Acuchimay near Ayacucho, *O. Tovar 2447* (USM). **Cajamarca:** Prov. Cajabamba, 6 km S of Cajabamba on rd. toward Huamachuco, *P. M. Peterson & N. F. Refulio-Rodriguez 13993* (US, USM); Prov. Cajamarca, just N of Colina, *P. Gutte & G. Muller 9093* (USM); 1 km S of Huambocancha on rd. toward Cajamarca, *P. M. Peterson & N. F. Refulio-Rodriguez 14845, 14847* (US, USM); 4 km N of San Juan on rd. to Cajamarca, *J. T. Columbus, J. M. Porter & E. H. Roalson 3511* (RSA, US); Prov. Cutervo, 13 km W of Cutervo on rd. toward Sócoto, *P. M. Peterson & N. F. Refulio-Rodriguez 15005* (US, USM). **Cusco:** Prov. Anta, near Chacán, *C. Vargas C. 017116* (CUZ); Prov. Cusco, Mishahuara, *C. Vargas C. 21997* (CUZ); Prov. Urubamba, Yahuarmaqui, *C. Vargas 9281* (US); Muyeh, *C. Vargas C. 014115* (CUZ). **Huancavelica:** Prov. Huancavelica, Izcuchaca, *O. Tovar 2447* (USM); Prov. Tayacaja, Hacienda Pilcos, below Colcabamba, *O. Tovar 1899* (US). **Huánuco:** Prov. Huánuco, near Huánuco, *O. Velarde 2546* (US). **Junín:** Prov. Huancayo, Huancayo en el Cerro La Libertad, *O. Velarde 2636* (US); Prov. Tarma, 1 km up rd. to Hacienda Maraynioc out of Palca, *P. M. Peterson & O. Tovar 14050* (US, USM). **Lima:** Prov. Lima, Dist. Lurín, *M. LaTorre 110* (USM).

11b. Eragrostis lurida subsp. **contracta** (Pilg.) P. M. Peterson & Sánchez Vega, stat. et comb. nov. Basionym: *Eragrostis contracta* Pilg., Bot. Jahrb. Syst. 37: 376. 1906. TYPE: Peru. Hacienda Cajabamba inter Samanco et Caraz, in formatione aperta, 3700 m, *A. Weberbauer 3035* (lectotype, designated here, US 2767408 [fragm.] ex B!). Figure 8D, E.

Panicles 0.5–2 cm, spicate, primary branches appressed and overlapping on the rachis.

Comments. The specimen *A. Weberbauer 3035* at US is chosen as lectotype because it is a fragment of a specimen from the original collection from B, which was destroyed during World War II.

Specimens examined. ECUADOR. **Chimborazo:** Alausi, *I. S. Hitchcock 20713* (US). **Cotopaxi:** 20 km N of Ambato & 21.5 km S of Latacunga, just off the Pan-American Hwy., *P. M. Peterson, C. R. Annable & M. Poston 8787* (US). **Pichincha:** Malchingui, *M. Acosta-Solís 16342* (US). **Tungurahua:** Ambato, *A. S. Hitchcock 21728* (US). PERU. Prope Ocros, in formatione aperta, *A. Weberbauer 2663* (syntype, US 1061508 [fragm.] ex B). **Ancash:** Prov. Bolognesi, 8 km E of Raquia & 2 km W of Cajacay on Ruta 02-104, *P. M. Peterson, N. F. Refulio-Rodriguez, A. Cano, M. LaTorre & I. Salinas 17876A* (US); Prov. Cajatambo, ad Chiquian, in formatione aperta, plantas herbaceas (maxime Gramineas) intermixtis fruticibus gerente, *A. Weberbauer 2841* (syntype, B not seen, BAA 1020 [fragm.] ex B). Prov. Recuay, Cordillera Blanca, 20 km E of Raquia on rd. toward Huaraz, *P. M. Peterson & N. Refulio R. 13794* (US, USM). **Arequipa:** Prov. Caylloma, 5 km W of Chivay on rd. toward Yanque, *P. M. Peterson & N. F. Refulio-Rodriguez 18296* (US, USM); Prov. Lucanas, 1 km S of Lucanas on rd. toward Puquio, *P. M. Peterson & N. F. Refulio Rodriguez 18200* (US, USM); Prov. Huanca Sancos, 27 km NW of Putajasa & 3 km S of Sacsamarca, *P. M. Peterson, A. Cano, M. LaTorre, A. Ramírez & D. Susanibar 16284* (US, USM). **Cajamarca:**

Figure 13. *Eragrostis lurida* subsp. *lurida* (*P. M. Peterson, E. J. Judziewicz & R. M. King 9103*; *P. M. Peterson & E. J. Judziewicz 9327*). —A. Habit. —B. Ligule. —C. Panicle branch. —D. Spikelet. —E. Lower glume (dorsal view). —F. Lower glume (ventral view). —G. Upper glume (dorsal view). —H. Upper glume (ventral view). —I. Rachilla with floret. —J. Lemma (ventral view). —K. Lemma (dorsal view). —L. Lemma (side view). —M. Palea (dorsal view). —N. Palea (ventral view). —O. Palea enclosing the stamens, pistil, and lodicules. —P. Palea enclosing the stamens, pistil, and lodicules with rachilla (side view). —Q. Stamens, pistil, and lodicules.

Figure 14. *Eragrostis magna* (*P. M. Peterson & N. F. Refulio-Rodriguez 16515*; *J. F. Macbride 4069*). —A. Habit. —B. Panicle. —C. Spikelet. —D. Floret.

Prov. Contumazá, Las Altamisas (Guzmango), *A. Sagástegui A. et al. 10382* (US). **Cusco:** Oyantaytambo, *A. S. Hitchcock 22486* (US). **Huancavelica:** Prov. Huancavelica, *O. Tovar 960* (US). **Junín:** Prov. Huancayo, Huancayo, *J. Soukup 1906* (US). **Lima:** Huarochirí, Tamboraque, *O. Velarde N. 5010* (US); Yauyos, Cruz Pampa, encima de Tupe, *E. Cerrate & O. Tovar 1109* (US). **Tacna:** Prov. Tarata, Tarata, *C. Vargas 9235* (US).

12. Eragrostis magna Hitchc., Contr. U.S. Natl. Herb. 24(8): 341. 1927. TYPE: Peru. Huánuco: Huacachi Estacion near Muna, 2000 m, 20 May–1 June 1923, *J. F. MacBride 4069* (holotype, F 535136 not seen; isotype, US 1256382!). Figures 5E–H; 14A–D.

Perennial, caespitose with innovations and short rhizomes near base; culms 80–150 cm tall, erect or ascending, robust, glabrous and somewhat shiny below the nodes, 3 or 4 nodes per culm, 4–8 mm in diam. at base. Leaf sheaths 2/3–3/4 the length of the internodes above, mostly glabrous or sparsely short-bearded at the summit; ligules ca. 0.25 mm, ciliate; blades (26–)40–60 × (0.3–)0.4–0.7 cm, flat below and in the middle, involute toward the apex, glabrous and pilose on the lower margins above. Panicles (22–)30–50 × 6–16 cm, narrowly ovate, somewhat condensed and densely flowered; primary branches 12–24 cm, semi-verticillate, ascending, not floriferous near base, branches diverging 0°–90° from the rachises; pulvini pilose, the hairs up to 3 mm; pedicels mostly 1–6 mm, erect, sinuous. Spikelets 5–8(–10) × 2–2.4 mm, (5 to)7- to 10-flowered, narrowly oblong to ovate-lanceolate, brownish olivaceous to plumbeous; disarticulation with the lemma first then the palea, rachilla mostly terete, persistent with a short crown of hairs at the apex, the hairs less than 0.2 mm; glumes 2–2.4 mm, subequal, hyaline, 1(3)-nerved, keeled, apex acute to acuminate; lower glume 2–2.2 mm, narrower than the upper; upper glume 2.1–2.4 mm; lemmas 2.4–2.9 mm, ovate to lanceolate, glabrous, membranous and hyaline on margins, margins involute on lower half, keeled toward apex, lateral nerves evident; apex acute to obtuse; paleae 2.4–2.9 mm, about as long as the lemma, hyaline, scaberulous along the keels; apex truncate; stamens 3, anthers 1.2–1.5 mm. Caryopses ca. 1.2–1.8 mm, rectangular-prismatic, strongly laterally flattened, striate and reticulate, with a deep ventral groove, narrowly triangular in cross section, reddish brown.

Distribution and habitat. Endemic to the central Peruvian Andes and known only from a few collections; occurs on steep, rocky, often calcareous slopes in grasslands; 1900–2670 m.

Phenology. Flowering March through June.

Specimens examined. PERU. **Apurímac:** Prov. Aymaráes, 16 km NW of Chalhuanca, *P. M. Peterson & N. F. Refulio-Rodriguez 16515* (US, USM). **Cajamarca:** Prov. Chota, Distrito Cochabamba, 4 km sobre la carretera Cochabamba–Cutervo, *I. Sánchez Vega 2292* (CPUN, US). **Huánuco:** Prov. Huánuco, Hacachi, near Muña, *J. F. Macbride 4069* (F, US).

13. Eragrostis maypurensis (Kunth) Steud., Syn. Pl. Glumac. 1: 276. 1854. Basionym: *Poa maypurensis* Kunth, Nov. Gen. Sp. 1: 161, 162. 1815 [1816]. *Megastachya maypurensis* (Kunth) Roem. & Schult., Syst. Veg. 2: 588. 1817. TYPE: Venezuela. Amazonas: Río Orinoco, Apr., *Humboldt & Bonpland s.n.* (holotype, P!; isotypes, BAA 1053 [fragm.]!, P!, US 2850758 [fragm.] ex P-Bonpl.!, [fragm.] ex B-W!). Figures 3I–L; 15A–C.

Annual, caespitose; culms 25–45 cm tall, erect to ascending, often decumbent, many branched from the base forming a rosette, mostly with 2 to 3(to 6) nodes. Leaf sheaths 1/2–2/3 as long as the internodes above, mostly glabrous, pilose at the summit and along upper margins; ligules ca. 0.5 mm, ciliate; blades 6–12 × 0.2–0.4 cm, flat to loosely involute toward the apex, scattered pilose near base and along margins to pilose abaxially and adaxially, the hairs 1.5–5 mm, papillose-based. Panicles 6–14 × (1.5–)3–7.5 cm, open, narrowly ovate to oblong, ± densely flowered, primary branches 1.5–5 cm, spreading, solitary at a node, floriferous to base, branches diverging 0°–70° from the rachises; pulvini pilose, the hairs up to 4 mm long; pedicels 0–1.5 mm, shorter than the spikelets, appressed, with a few scattered hairs. Spikelets 8–15(–30) × 1.8–2.5(–3) mm, 12- to 35-flowered, narrowly lanceolate to oval-lanceolate, florets imbricate, reddish purple to yellowish; disarticulation acropetal with the paleae and glumes persistent; glumes (1.5–)2–2.6 mm, subequal, lanceolate-acuminate, strongly to weakly keeled, scaberulous along the keel; lower glumes 2–2.6 mm, usually longer than the upper; upper glumes (1.5–)2–2.6 mm; lemmas 1.8–3 mm, ovate-acuminate, chartaceous, lateral nerves and midnerve evident, usually green; paleae 1.5–2.2 mm, shorter than the lemma, hyaline; stamens 2, anthers 0.2–0.3 mm, purplish. Caryopses 0.4–0.7 mm, ovoid, translucent, rhomboid reticulate without striations, obovate to circular in cross section, reddish brown. $2n = 20$ (Pohl & Davidse, 1971).

Distribution and habitat. Native to the Americas from Mexico, Central America to South America in Bolivia, Brazil, Colombia, Peru, and Venezuela (Nicora, 1998; Laegaard & Peterson, 2001); a fairly

Figure 15. *Eragrostis maypurensis* (*J. R. Swallen 3306*). —A. Habit. —B. Spikelet. —C. Floret. *Eragrostis pilgeri* subsp. *pilgeri* (*P. M. Peterson & N. F. Refulio-Rodriguez 13919*). —D. Spikelet. —E. Floret. —F. Lemma (dorsal view). —G. Palea enclosing the stamens, pistil, and lodicules.

common grass in the Amazonian region where it is found growing in savannahs, sandy sites, along roadsides, and disturbed areas: 100–1000 m.

Phenology. Flowering September through May.

Specimens examined. PERU. **Madre de Dios:** Prov. Tambopata, Puerto Maldonado, entre Aeropuerto Viejo y Puerto Maldonado, *P. J. Babour 5396* (AMAZ). **San Martín:** Alto Río Huallaga, *L. Williams 5791* (US); Dist. San Martín, along Río Shilcayo, 1–4 km NE of Tarapoto, *C. M. Belshaw 3359* (F, MICH, MO, UC, US, WIS).

14. Eragrostis mexicana (Hornem.) Link, Hort. Berol. 1: 190. 1827. Basionym: *Poa mexicana* Hornem., Hort. Bot. Hafn. 2: 953. 1815. TYPE: Mexico. Cultivated from seed collected in Mexico, *Sessé s.n.* (lectotype, designated here, MA!).

Caespitose annuals; culms 10–130 cm tall, erect, sometimes geniculate, glabrous, sometimes with a ring of glandular depressions below the nodes. Leaf sheaths 1/2–2/3 as long as the internodes, sometimes with glandular pits, pilose near the apices and on the collars, hairs to 4 mm, papillose-based; ligules 0.2–0.5 mm, ciliate; blades 5–25 × 0.2–0.7(–0.9) cm, flat, abaxial surfaces glabrous, adaxial surfaces scabridulous, occasionally pubescent near the base. Panicles (5–)10–40 × (2–)4–18 cm, less than 1/2 the height of the plant, ovate, rachises angled and channeled; primary branches 3–12(–15) cm, solitary to whorled, appressed or diverging to 80° from the rachises; secondary branches somewhat appressed; pulvini glabrous; pedicels 1–6(–7) mm, almost appressed to narrowly divergent, stiff. Spikelets (4–)5–10(–11) × 0.7–2.4 mm, 5- to 11(to 15)-flowered, linear to linear-lanceolate or ovate to oblong, gray-green to purplish; disarticulation acropetal; glumes 0.7–2.3 mm, subequal, ovate to lanceolate, membranous; lemmas 1.2–2.4 mm, ovate, membranous, glabrous or with a few hairs, gray-green, lateral veins evident, often greenish; apex acute; paleae 1–2.2 mm, hyaline, keels scabrous; apex obtuse to truncate; stamens 3; anthers 0.2–0.5 mm, purplish. Caryopses 0.5–0.8(–1) mm, ovoid to rectangular-prismatic, laterally flattened, shallowly to deeply grooved on the ventral surface, striate and reticulate, irregularly triangular in cross section, reddish brown, distal 2/3 opaque. $2n = 60$ (Pohl & Davidse, 1971).

Distribution and habitat. *Eragrostis mexicana* is native to the Americas and grows along roadsides, near cultivated fields, and in disturbed open areas, in lomas vegetation and slopes of the inter Andes of Peru; 60–3900 m. The species has been treated as two distinct subspecies by Koch and Sánchez-Vega (1985), both of which occur in Peru.

Phenology. Flowering September through June.

Comments. The specimen *Sessé s.n.* at MA is chosen as lectotype because it is located at the institution where the author worked and deposited types.

Specimens examined. BRAZIL. *Sessé & M. Lacasta s.n.* (syntype, US 2891498 [fragm.]).

KEY TO THE SUBSPECIES OF *ERAGROSTIS MEXICANA*

14a. Spikelets ovate to oblong in outline, 1.5–2.4 mm wide; lower glumes 1.2–2.3 mm long; sum of the spikelet width and lower glume length 2.7–4.7 mm; culms and sheaths sometimes with glandular depressions . . . . . *E. mexicana* subsp. *mexicana*

14b. Spikelets linear to linear-lanceolate, 0.7–1.4 mm wide; lower glumes 0.7–1.7 mm long; sum of the spikelet width and lower glume length 1.5–3.1 mm; culms and sheaths without glandular depressions . . . . . *E. mexicana* subsp. *virescens*

14a. Eragrostis mexicana (Hornem.) Link subsp. **mexicana**. Figures 5I–L; 16A–C.

Eragrostis limbata E. Fourn., Mexic. Pl. 2: 116. 1886. TYPE: Mexico. 1833, *A. J. A. Bonpland 4573* (lectotype, designated by McVaugh, Fl. Novo-Galic. 14: 168. 1983, P!; isotype, US 2941517 [fragm.]!).

Eragrostis neomexicana Vasey ex L. H. Dewey, Contr. U.S. Natl. Herb. 2(3): 542. 1894. TYPE: U.S.A. New Mexico: Organ Mountains, 1881, *G. Vasey 474* (lectotype, designated by Koch & Sánchez Vega, Phytologia 58: 379. 1985, US 176631!; isotypes, K!, US 822049!, US 909912!).

Eragrostis alba J. Presl, Reliq. Haenk. 1(4–5): 279. 1830. *Poa alba* (J. Presl) Kunth, Enum. Pl. 1: 343. 1833. TYPE: Peru. *T. Haenke s.n.* (holotype, PR not seen; isotypes, MO 2111118!, US 2942409 [fragm.] ex P!).

Culms and sheaths sometimes with glandular depressions. Spikelets 1.5–2.4 mm, ovate to oblong; lower glumes 1.2–2.3 mm; sum of spikelet width and lower glume length 2.7–4.7 mm.

Comments. McVaugh did not indicate the herbarium when he designated *A. J. A. Bonpland 4573* as lectotype (McVaugh, 1983), but Bonpland's collections are housed at P.

Specimens examined. PERU. **Ancash:** Prov. Bolognesi, 8 km E of Raquia & 2 km W of Cajacay on Ruta 02-104, *P. M. Peterson, N. F. Refulio-Rodriguez, A. Cano, M. LaTorre & I. Salinas 17885* (US, USM); Prov. Huaraz, near Huaraz, *F. Castillo C. & Nuñez L. s.n.* (HUT). **Arequipa:** Tingo, *F. W. Pennell 13109* (US). **Cajamarca:** Prov. Cutervo, Sócota, camino a San Andrés, *I. Sánchez-Vega 2301* (CPUN). **Junín:** Prov. Chanchamayo, Río Rondayacu, 45 km from San Ramón, *D. N. Smith et al. 2622* (US). **La Libertad:** Prov. Trujillo, Barraza, *A. Sagástegui A. 7789* (US). **Lima:** Prov. Canta, 2 km SE of San Jose Canta, *P. M. Peterson & N. F. Refulio Rodriguez 17987* (US, USM); Lurín, *J. F. Macbride 5954* (US).

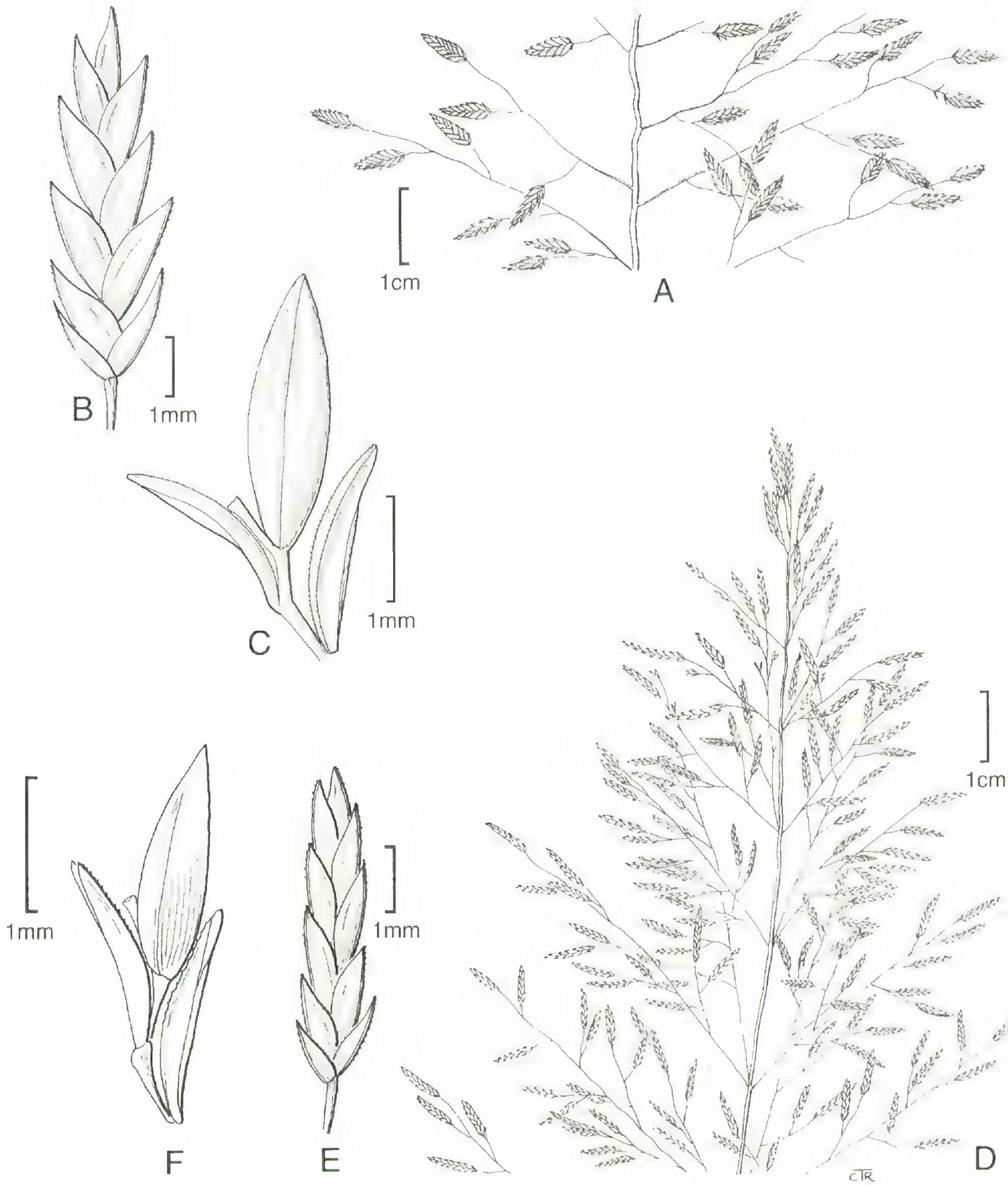

Figure 16. *Eragrostis mexicana* subsp. *mexicana*. —A. Paniele. —B. Spikelet. —C. Rachilla with paleae (right and left) and lemma (right). *Eragrostis mexicana* subsp. *virescens*. —D. Paniele. —E. Spikelet. —F. Rachilla with paleae (right and left) and lemma (right).

14b. Eragrostis mexicana subsp. **virescens** (J. Presl) S. D. Koch & Sánchez Vega, Phytologia 58(6): 380. 1985. Basionym: *Eragrostis virescens* J. Presl, Reliq. Haenk. 1(4–5): 276. 1830. TYPE: Chile. *T. Haenke s.n.* (holotype, PR not seen; isotypes, B!, BAA 1107 [fragm.] ex B!, LE-TRIN 2413.01!, US 2942410 [fragm.]!). Figures 5M–P; 16D–F.

Culms and sheaths without glandular depressions. Spikelets 0.7–1.4 mm, linear to linear-lanceolate; lower glumes 0.7–1.7 mm; sum of spikelet width and lower glume length 1.5–3.1 mm.

Distribution and habitat. Native to South America in Argentina, Bolivia, Brazil, Chile, Paraguay, Uruguay; introduced in Canada, Mexico, and U.S.A. (Nicora, 1998; Peterson & Boechat, 2001).

Specimens examined. PERU. **Ancash:** Fortaleza, *E. Anderson 464* (US). **Apurímac:** Prov. Abancay, 17 km NE of Abancay & 16 km W of Río Apurímac, *P. M. Peterson & N. F. Refulio-Rodriguez 16530* (US, USM); Prov. Aymaráes,

16 km NW of Chalhuanca, *P. M. Peterson & N. F. Refulio-Rodriguez 16514* (US, USM). **Arequipa:** Prov. Arequipa, Tiabaya, *F. W. Pennell 13152* (US); along Hwy. (Carretera Panamericana) 9.3 km SE of bridge over Río Síhuas, *J. T. Columbus, J. M. Porter & E. H. Roalson 3526* (RSA, US); Prov. Condesuyos, below Chuquibamba, *C. Vargas C. 019413* (CUZ); Prov. La Unión, below Cotawasi, *C. Vargas C. 019492* (CUZ). **Cajamarca:** Prov. Cajamarca, 40 km N of Cajabamba & 13 km S of Ichocan, *P. M. Peterson & N. F. Refulio-Rodriguez 14008* (US, USM). **Cusco:** Prov. Convención, Valle de Lucumayo, *C. Vargas C. 020025* (CUZ); Prov. Urubamba, Urquillos–Urubamba, *C. Vargas C. 1213* (CUZ). **La Libertad:** Prov. Trujillo, Cerro Campana, ca. 15 km al N de Trujillo, *M. O. Dillon 2731* (US). **Lima:** Prov. Canta, 8 km SW of San Jose Canta toward Huamantango, *P. M. Peterson & N. F. Refulio Rodriguez 17994* (US, USM); Huaura Valley, 20 km below Churín, *E. Anderson 595* (US). **Piura:** Km 988 Panamericana, *E. Anderson 925* (US). **San Martín:** Prov. Rioja, at Puente de Rio Serranoyacu, Km 405–406, *J. L. Luteyn, I. Sánchez-Vega & M. Zapata Cruz 15518* (NY, US). **Tacna:** Prov. Tacna, *C. Vargas C. 018075* (CUZ).

15. Eragrostis nigricans (Kunth) Steud., Nom. Bot. ed. 2, 1: 563. 1840. Basionym: *Poa nigricans* Kunth, Nov. Gen. Sp. 1: 159. 1816. *Megastachya nigricans* (Kunth) Roem. & Schult., Syst. Veg. 2: 586. 1817. TYPE: Ecuador. Chillo & Sangolqui, Apr.–May, *F. Humboldt & A. Bonpland 2291* (holotype, P-Bonpl.!; isotypes, BAA 1062 [fragm.]!, K photo!, LE-TRIN 2371.01!, P!, US 2891495 [fragm.] ex LE-TRIN!, [fragm.] ex P!). Figures 1M–P; 10D–F.

Eragrostis scabra Phil., Fl. Atacam. 55. 1860. TYPE: Chile. Atacama: prope Paposo, Dec. 1851, *R. A. Philippi 1051* (holotype, SGO-PHIL 357 not seen; isotypes, B!, BAA 1089 [fragm.] ex B!, US 556539 [fragm.] ex SGO-PHIL 357! & photo!).

Eragrostis rahmeri Phil., Verz. Antofagasta Pfl. 88. 1891. TYPE. Chile. Tarapacá: Quebrada de Guaviña, 13 Mar. 1885, *R. A. Philippi s.n. 359* (holotype, SGO-PHIL 359 not seen; isotypes, BAA 1080 [fragm.]!, CORD!, SGO 37293 not seen, SGO 63537 not seen, SGO 62670 not seen, US 556538 [fragm.] ex SGO-PHIL 359!).

Eragrostis tristis Jedwabn., Bot. Arch. 5(3–4): 205. 1924. *Eragrostis nigricans* var. *tristis* (Jedwabn.) Pilg., Notizbl. Bot. Gart. Berlin-Dahlem 11: 778. 1933. TYPE: Bolivia. Ad Huancapamba, 15 Feb. 1910, *Pflanz 359* (lectotype, designated here, BAA 1101 [fragm.] ex B!).

Eragrostis subatra Jedwabn., Bot. Arch. 5(3–4): 202. 1924. TYPE: Bolivia. Prope La Paz, 1889, *M. Bang 80* [collector erroneously cited as Rusby in protologue] (holotype, B not seen; isotypes, K!, US 822065!).

Annual, caespitose; culms (10–)20–50(–80) cm tall, erect, sometimes geniculate below, mostly glabrous and somewhat shiny below the nodes. Leaf sheaths 1/3–2/3 as long as the internodes, sparsely pilose at the summit and along the upper margins, the hairs up to 2.5 mm, sometimes the margins glabrous; ligules 0.4–0.6 mm, ciliate; blades 6.5–10 × 0.2–0.5 cm, flat, occasionally loosely involute near apex, glabrous above and below, somewhat scaberulous near apex. Panicles (5–)7–24 × 2–7(–15) cm, oblong, somewhat condensed, spikelets arranged in glomerules that are widely spaced along the channeled rachis, primary branches mostly 1–10 cm, sinuous, ascending and spreading 20°–90° from the rachises, solitary to whorled below, scaberulous, secondary branches sinuous; pulvini glabrous to sparsely pilose, the hairs up to 1.5 mm; pedicels 0.4–2(–3) mm, stiffly spreading, divaricate and stout, scaberulous. Spikelets 2.6–3.8(–4.8) × 1–1.2 mm, 2- to 4(to 5)-flowered, linear to narrowly lanceolate, grayish green to purplish green; disarticulation acropetal, with the glumes first, then the lemmas falling, paleae persistent; glumes 1–1.4 mm, subequal, ovate to lanceolate, membranous, keeled, scaberulous along the keel; lemmas 1.6–2 mm, ovate, membranous; grayish green, glabrous or with a few scattered hairs, lateral nerves sometimes distinct; apex acute, sometimes scaberulous; paleae 0.9–1.4 mm, hyaline, scaberulous along the keels; apex truncate; stamens 3, anthers 0.3–0.5 mm, purplish. Caryopses (0.6–) 0.7–1.1 mm, ovoid, striate and reticulate, shallowly to deeply grooved on the ventral surface, translucent, irregularly rectangular in cross section, reddish brown.

Distribution and habitat. Native to the Andean mountains in Argentina, Bolivia, Chile, Ecuador, and Peru (Nicora, 1998; Peterson & Boechat, 2001); occurs on rocky slopes, near cultivated fields, and disturbed roadsides; 1000–4000 m.

Phenology. Flowering September through April.

Comments. For *Eragrostis tristis*, the specimen *Pflanz 359* at BAA, which is a fragment from the destroyed B collection, is chosen as lectotype because the author, Elizabeth Jedwabnick, most likely worked with the specimens that were housed at B.

Specimens examined. PERU. **Ancash:** Prov. Huari, Cordillera Blanca, 21 km S of Huari on rd. toward San Marcos, *P. M. Peterson & N. F. Refulio-Rodriguez 13855* (US, USM); 4 km W of Ponto on rd. toward Palca, *P. M. Peterson, N. F. Refulio-Rodriguez, A. Cano, M. LaTorre & I. Salinas 17927* (US, USM). **Apurímac:** Prov. Abancay, 17 km NE of Abancay & 16 km W of Rio Apurímac, *P. M. Peterson & N. F. Refulio-Rodriguez 16534* (US, USM); Prov. Aymaráes, ca. al puente Cuícua, *O. Velarde 5688* (US); 16 km NW of Chalhuanca, *P. M. Peterson & N. F. Refulio-Rodriguez 16509* (US, USM); 24 km NW of Chalhuanca, *P. M. Peterson & N. F. Refulio-Rodriguez 16526* (US, USM). **Arequipa:** Prov. Arequipa, 21 km N of Yura on hwy. toward Patahuasí & 45 km NW of Arequipa, *P. M. Peterson & N. F. Refulio-Rodriguez 18235* (US, USM); S slope of Nevado Chachani, *J. T. Columbus, J. M. Porter & E. H. Roalson 3538* (RSA, US); Prov. Caravelí, Lomas Jahuay, ca. 33 km WNW from Chavina, *M. O. Dillon, U. Molau & P. Matekaitis 3258* (CPUN); Prov. Caylloma, 5 km W of Chivay on rd. toward

Yanque, *P. M. Peterson & N. F. Refulio-Rodriguez 18294* (US, USM). **Ayacucho:** Prov. Ayacucho, *J. Soukup 5111* (US); Prov. Lucanas, 6 km E of Puquio on rd. toward Chaviña, *P. M. Peterson, M. LaTorre, A. Ramírez & D. Susanibar 16291* (US, USM). **Cajamarca:** Prov. Cajamarca, ca. a la localidad de Jesús, *I. Sánchez Vega 1061* (CPUN); 6 km S of Cajabamba on rd. toward Huamachuco, *P. M. Peterson & N. F. Refulio-Rodriguez 13995* (US, USM); Prov. Cutervo, entre San Andrés y Sócota, *J. Mostacero et al. 1713* (HUT); 13 km W of Cutervo on rd. toward Sócoto, *P. M. Peterson & N. F. Refulio-Rodriguez 15009* (US, USM). **Cusco:** Prov. Urubamba, Yahuarmaqui, *C. Vargas 009287* (US). **Huancavelica:** Entre Pampas y Salcabamba, *O. Torar 3854* (US). **Huánuco:** Prov. Huanuco, Cayumba, *E. Asplund 13451* (US). **Ica:** 32 km E of Nasca on rd. toward Puquio, *P. M. Peterson & N. F. Refulio-Rodriguez 16424* (US, USM). **Junín:** Prov. Tarma, 1 km up rd. to Hacienda Maraynioc at Palca, *P. M. Peterson & N. F. Refulio-Rodriguez 14051* (US, USM). **La Libertad:** Prov. Santiago de Chuco, 22 km E from Huamachuco on rd. toward Sarín, *P. M. Peterson & N. F. Refulio-Rodriguez 13979* (US). **Lima:** Prov. Canta, 2 km SE of San Jose Canta, *P. M. Peterson & N. F. Refulio-Rodriguez 17986* (US, USM); Prov. Lima, Huaura Valley, 10 km below Churín, *E. Anderson 599* (US). **Moquegua:** Torata, *A. Weberbauer 7405* (US). **Piura:** Prov. Huancabamba, 10 km N of Sondor & 3 km S of Huancabamba, *P. M. Peterson & N. F. Refulio-Rodriguez 15166* (US, USM). **Tacna:** Prov. Tarata, Dist. Tarata, *C. Vargas C. 009228* (CUZ); Candarave, *A. Weberbauer 7384* (US).

16. Eragrostis pastoensis (Kunth) Trin., Mém. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat. 4(2): 71. 1836. Basionym: *Poa pastoensis* Kunth, Nov. Gen. Sp. 1: 160. 1815 [1816]. *Megastachya pastoensis* (Kunth) Roem. & Schult., Syst. Veg. 2: 587. 1817. TYPE: Colombia. Pasto, Mt. Arand, *F. Humboldt & A. Bonpland 2149* (holotype, P-Bonpl.!; isotypes, P!, P photo at K!, US 2767397 [fragm.] ex P-Bonpl.!). Figures 1E–H; 17A–P.

Poa montufari Kunth, Nov. Gen. Sp. 1: 159. 1815 [1816]. *Eragrostis montufari* (Kunth) Steud., Nom. Bot. (ed. 2) 1: 563. 1840. *Megastachya montufari* (Kunth) Roem. & Schult., Syst. Veg. 2: 586. 1817. TYPE: Ecuador. Pichincha: betw. Puembo & San Antonio de Lulumbamba, Apr.–May, *F. Humboldt & A. Bonpland s.n.* (holotype, P-Bonpl.!; isotypes, BAA 1056!, P!, US 2891497! [fragm.] ex P-Bonpl.!).

Poa olmedoi Kunth, Nov. Gen. Sp. 1: 159–160. 1815 [1816]. *Megastachya olmedoi* (Kunth) Roem. & Schult., Syst. Veg. 2: 586. 1817. *Eragrostis olmedoi* (Kunth) Steud., Nom. Bot. (ed. 2) 1: 564. 1840. TYPE: Peru. July, *F. Humboldt & A. Bonpland s.n.* (holotype, P!).

Poa tenax Kunth, Nov. Gen. Sp. 1: 160. 1815 [1816]. *Eragrostis tenax* (Kunth) Steud., Nom. Bot. (ed. 2) 1: 564. 1840. *Megastachya tenax* (Kunth) Roem. & Schult., Syst. Veg. 2: 587. 1817. TYPE: Ecuador. Apr.–May, *F. Humboldt & A. Bonpland s.n.* (holotype, P!; isotype, US 2891490 [fragm.] ex P-Bonpl.!).

Poa setifolia Benth., Pl. Hartw. 262. 1846. *Eragrostis setifolia* (Benth.) Steud., Syn. Pl. Glumac. 1: 274. 1854, nom. illeg. hom. TYPE: Ecuador. Quito, *Hartweg 1452* (holotype, K!; isotypes, US 2826937 [fragm.] ex K!, US 2850739 [fragm.] ex CGE!, [fragm.] ex P!, [fragm.] ex P-STEUD!).

Eragrostis lehmannii Pilg., Bot. Jahrb. Syst. 27(1–2): 32. 1899. TYPE: Ecuador. ca. Baños ad flumen Tunguragua, 1800–2500 m, *F. C. Lehmann 5283* [error for *5823*] (isotype, US 2767406 [fragm.]!).

Eragrostis virescens var. *trachyphylla* Hack., Anales Mus. Nac. Buenos Aires 13: 505. 1906. TYPE: Argentina. Tucuman: *Stuckert herb. arg. 14866 ex Lillo Herb. arg. 3192* (holotype, W not seen; isotype, US 2942411 [fragm.]!).

Eragrostis buchtienii Hack., Repert. Spec. Nov. Regni Veg. 6: 157. 1908. TYPE: Bolivia. Sud Yungas: Simpayas bei Yanacachi, *O. Buchtien 428* (holotype, W not seen; isotype, US 77388!).

Perennial, caespitose, with innovations; culms 30–90 cm tall, erect, sometimes geniculate below, glabrous and somewhat shiny below the nodes. Leaf sheaths overlapping below, 3/4 as long as the internodes above, ciliate at the summit and along the upper margins; ligules 0.2–0.5 mm, ciliate; blades 5–40(–45) × 0.1–0.4(–0.7) cm, involute or flat, glabrous to scaberulous below and scaberulous above, sometimes with scattered hairs, the hairs up to 4 mm. Panicles 8–45 × 1–27 cm, ovate, lanceolate to narrowly oblong, contracted or open, rachis glabrous, ascending primary branches 1–20 cm, appressed to widely spreading up to 80 from the rachises, the branches glabrous to scaberulous, not floriferous near base; secondary branches composed of loosely overlapping spikelets; pulvini ciliate or glabrous; pedicels 0.5–5 mm, erect, mostly appressed, sometimes with hairs. Spikelets 2–6 × 1–1.8 mm, 2- to 8-flowered, lanceolate to oblong-ovate, inflated to slightly compressed, plumbeous, sometimes purple-tinged, rachilla sometimes sparingly ciliate; disarticulation acropetal, with the glumes first then the lemmas falling individually, paleae persistent; glumes 1–1.9 mm, subequal, broadly ovate to lanceolate, membranous, subhyaline, keeled, scaberulous along the keel; lower glume 1–1.4 mm, narrow lanceolate; upper glume 1.2–1.9 mm, usually broader than the lower; lemmas 1.2–2(–2.1) mm, ovate to broadly ovate, membranous, lateral nerves obscure, keeled, especially toward the apex, scaberulous along the keel; apex acute; paleae 1–2 mm, membranous to partially hyaline, scaberulous along the keels; apex obtuse to truncate; stamens 3, anthers 0.3–0.6 mm, reddish purple. Caryopses 0.4–0.9 mm, obovoid to prism-shaped, striate and reticulate, usually with a ventral groove, irregularly rectangular in cross section, light reddish brown to translucent. $2n = 70$ (Bowden & Senn. 1962, as *Eragrostis montufari*).

Figure 17. *Eragrostis pastoensis* (*P. M. Peterson, C. R. Annable & M. E. Poston 8910*; *P. M. Peterson & E. J. Judziewicz 9268*). —A. Habit. —B. Ligule. —C. Portion of the panicle. —D. Spikelet. —E. Lower glume (dorsal view). —F. Lower glume (ventral view). —G. Upper glume (dorsal view). —H. Upper glume (ventral view). —I. Rachilla with floret. —J. Lemma (dorsal view). —K. Lemma (ventral view). —L. Palea enclosing the stamens and lodicules (side view). —M. Palea enclosing the stamens, pistil, and lodicules. —N. Palea enclosing the pistil and lodicules. —O. Stamens and lodicules. —P. Pistil.

Distribution and habitat. Native to South America in Argentina, Bolivia, Brazil, Colombia, Ecuador, Paraguay, Peru, Uruguay, and Venezuela (Nicora, 1998; Peterson & Boechat, 2001); occurs on dry rocky hillsides, slopes, pastures, roadsides, and barrancas from 1400–3600 m.

Phenology. Flowering December through June.

Specimens examined. PERU. **Amazonas:** Prov. Bongará, 1 km up Shipasbamba rd. from Camp. Ingenio, *P. C. Hutchinson & J. K Wright 3963* (US). **Ancash:** Prov. Huari, Cordillera Blanca, 21 km S from Huari on rd. toward San Marcos at Río Mosna, *P. M. Peterson & N. F. Refulio-Rodriguez 13859* (US, USM). **Apurímac:** Prov. Abancay, near Abancay Quisapata, *C. Vargas C. 008899* (CUZ); Prov. Abancay/Andahuaylas, 4.8 km SW of Abancay on rd. to Andahuaylas, *P. M. Peterson & N. F. Refulio-Rodriguez 16649* (US, USM); Prov. Aymaráes, 9 km SW of Cotaruse on rd. toward Puquio, *P. M. Peterson & N. F. Refulio-Rodriguez 16481* (US, USM); 21 km NW of Chalhuanca, *P. M. Peterson & N. F. Refulio-Rodriguez 16522*. **Ayacucho:** Prov. Huamanga, Ayacucho, *O. Velarde 6231* (US); 25 Mar. 1946, *C. Vargas C. 5981* (CUZ). **Cajamarca:** Prov. Cajamarca, Cerro Huacarís, Valle de Cajamarca, *I. Sánchez Vega 747* (CPUN); 1 km S of Huambocancha, *P. M. Peterson & N. F. Refulio-Rodriguez 14846*; Prov. Chota, 7 km N of Chota on rd. toward Conchan, *P. M. Peterson & N. F. Refulio-Rodriguez 14974* (US, USM); Prov. Contumazá, Corlás, Cascas -Contumazá, *A. Sagástegui & S. Leiva G. 15514* (HAO); Prov. Cutervo, near Muñuño, *S. Llatas Q. 89* (HUT); 13 km W of Cutervo on rd. toward Sócoto, *P. M. Peterson & N. F. Refulio-Rodriguez 15006* (US, USM). **Cusco:** Prov. Urubamba, 20 km N of Ollantaytambo, *A. S. Hitchcock 22522* (US). **Huancavelica:** Mejorada, entre Izcuchaca y Acoria, *O. Tovar 2455* (US, USM). **Junín:** Prov. Oxapampa, Oxapampa, *O. Velarde 5427* (US). **La Libertad:** Prov. Santiago de Chuco, 22 km E from Huamachuco on rd. toward Sarín, above Río Chusgón, *P. M. Peterson & N. F. Refulio-Rodriguez 13982* (US, USM). **Piura:** Prov. Huancabamba, along rd. to Jaén & Moyobamba, *J. T. Columbus, J. M. Porter & E. H. Roalson 3458* (RSA, US); 14 km E of Sondor on rd. toward Tabaconas, *P. M. Peterson & N. F. Refulio-Rodriguez 15161* (US, USM).

17. Eragrostis pectinacea (Michx.) Nees var. **pectinacea**, Fl. Afr. Austral. Ill. 406. 1841. Basionym: *Poa pectinacea* Michx., Fl. Bor.-Amer. 1: 69. 1803. TYPE. U.S.A. Illinois: *Michaux s.n.* (holotype, P-MICH!; isotype, US 2851264 [fragm.] ex P!). Figures 6M–P; 18A–C.

Eragrostis diffusa Buckley, Proc. Acad. Nat. Sci. Philadelphia 14: 97. 1862. *Eragrostis purshii* var. *diffusa* (Buckley) Vasey, Contr. U.S. Natl. Herb. 1(2): 59. 1890. TYPE: U.S.A. Texas. *S. B. Buckley s.n.* (lectotype, designated by Hitchcock, Man. Grasses U.S. 849. 1935 [without citing a specific sheet or a specific herbarium], PH not seen; isotype, US 91621!).

Annuals, caespitose, without glandular pits; culms 10–80 cm tall, erect to geniculate or decumbent below, glabrous. Leaf sheaths overlapping below, 1/2–3/4 as long as the internodes above, hirsute at the apices, hairs to 4 mm long; ligules 0.2–0.5 mm; blades 2–20 × 0.1–0.45 cm, flat to involute, abaxial surfaces glabrous and smooth, adaxial surfaces scabridulous. Panicles 5–25 × 3–12(–15) cm, ovoid to pyramidal, usually open, sometimes contracted, primary branches 0.6–8.5 cm, appressed or slightly diverging to 20° from the rachises, solitary or paired at the lowest 2 nodes; pulvini glabrous or sparsely hairy; pedicels 1–7 mm, flexible, appressed to widely divergent, sometimes capillary. Spikelets 3.5–11 × 1.2–2.5 mm, 6- to 22-flowered, linear-oblong to narrowly lanceolate, plumbeous, yellowish brown, or dark reddish purple; disarticulation acropetal, paleae persistent; glumes 0.5–1.7 mm, subequal, subulate to ovate-lanceolate, hyaline; lower glumes 0.5–1.5 mm, at least 1/2 as long as the adjacent lemmas; upper glumes 1–1.7 mm, usually broader than the lower glumes; lemmas 1–2.2 mm, ovate-lanceolate, hyaline to membranous, grayish green proximally, reddish purple distally, lateral veins moderately conspicuous; apex acute; paleae 1–2 mm, hyaline to membranous, keels scabridulous; apex obtuse; stamens 3; anthers 0.2–0.4 mm, purplish. Caryopses 0.5–1.1 mm, rectangular-prismatic, slightly laterally flattened, striate and reticulate, rectangular with nearly equal sides in cross section, brownish. $2n = 60$ (Koch, 1974; Davidse, 1981).

Distribution and habitat. Native to the Americas; found in North America, Central America, the Caribbean, and most of South America (not known from Chile and Colombia); it grows in disturbed sites such as roadsides, railroad embankments, gardens, and cultivated fields; 150–2500 m (Peterson & Boechat, 2001).

Phenology. Flowering February through April.

Specimens examined. PERU. **Arequipa:** Prov. Caravelí, Lomas de Jahuay, 33 km NW de Chaviña, *M. Dillion, U. Molau & P. Matekaitis 3259* (CPUN, F). **La Libertad:** Prov. Santiago de Chuco, 22 km E of Huamachuco on rd. toward Sarín, above Río Chusgón, *P. M. Peterson & N. F. Refulio-Rodriguez 13981* (US, USM). **Lambayeque:** Prov. Lambayeque, Olmos, N side of Río Olmos, *J. T. Columbus, J. M. Porter & E. H. Roalson 3451* (RSA, US).

18. Eragrostis peruviana (Jacq.) Trin., Mém. Acad. Imp. Sci. St.-Pétersbourg, Sér. 6, Sci. Math. 1(4): 396. 1830. Basionym: *Poa peruviana* Jacq., Collectanea 1: 107. 1786 [1787]. TYPE: Peru. *N. J. von Jacquin* (holotype, W not seen; isotype, LE not seen, US 2851260 [fragm.]!). Figures 3M–P; 19A–C.

Koeleria multiflora Regel & Herter, Index Sem. (St. Petersburg) 23. 1858. TYPE: Chile. Cultivated seed from Chile, *M. Philippe* s.n. (holotype, LE not seen).

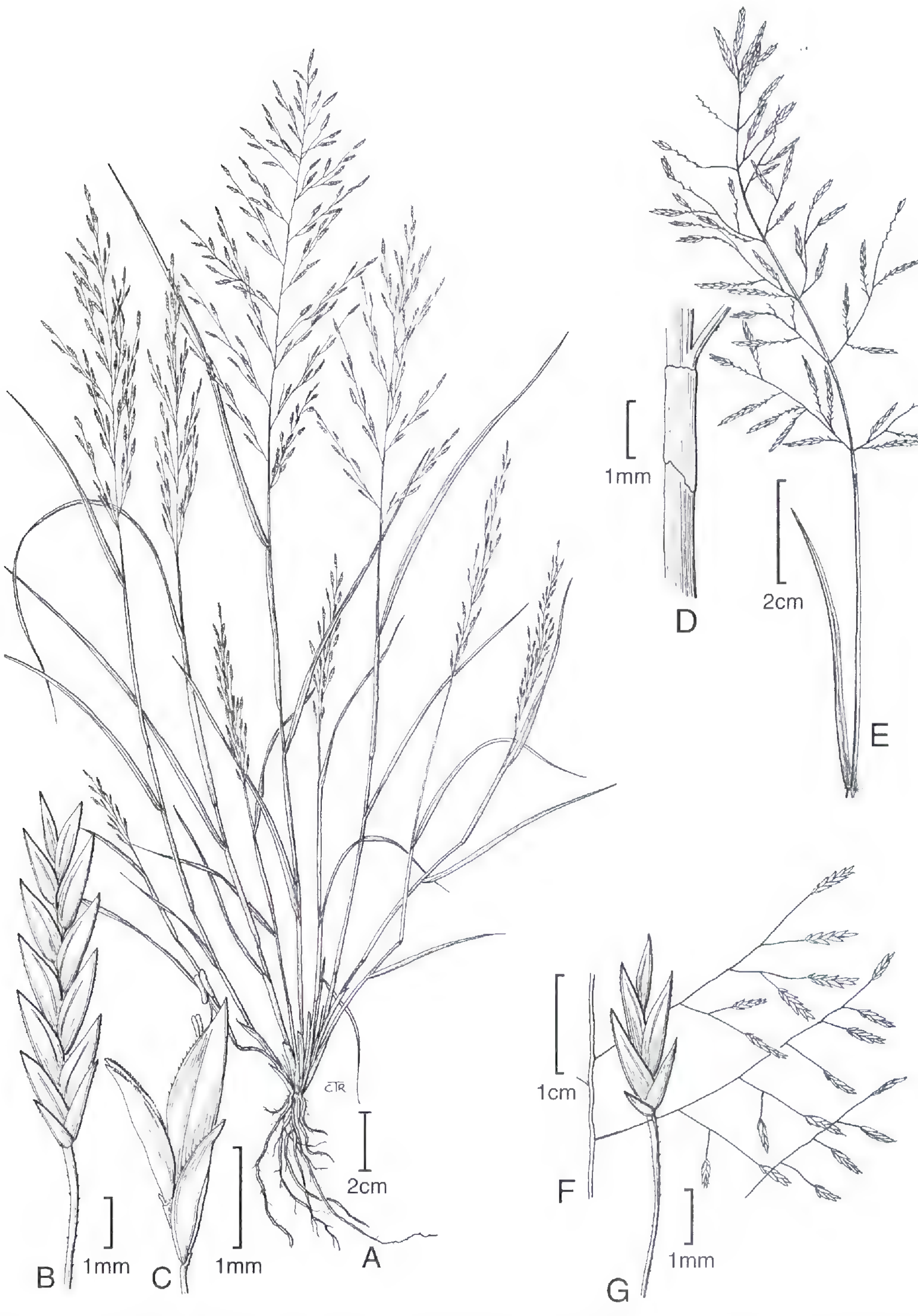

Figure 18. *Eragrostis pectinacea*. —A. Habit. —B. Spikelet. —C. Rachilla with three florets. *Eragrostis pilosa*. —D. Culm rachis showing glandular band. —E. Panicle with flag leaf. —F. Panicle. —G. Spikelet.

Eragrostis deserticola Phil., Fl. Atacam. 55. 1860. TYPE: Chile. Atacama: ca. Hueso Parado, 1200 m, *R. A. Philippi 409* (holotype, SGO-PHIL 358 not seen: isotypes, BAA [fragm.]!, SGO 63452 not seen, US 556537 [fragm.] ex SGO-PHIL 358!).

Eragrostis peruviana var. *brachythyrsa* Pilg., Bot. Jahrb. Syst. 37: 375. 1906. TYPE: Peru. Mollendo, in formatione "loma," 200–600 m, Oct. 1902, *A. Weberbauer 1462* (lectotype, designated by Nicora [as "holotypus"], Gayana, Bot. 51: 2. 1994, B not seen; isotype, US 2851259 [fragm.]!).

Annual, caespitose; culms (8–)27–37 cm tall, erect to prostrate, geniculate and many branched

Figure 19. *Eragrostis peruviana* (*M. O. Dillon, U. Molau & P. Matekaitis 3314*). —A. Habit. —B. Spikelet. —C. Floret. *Eragrostis weberbaueri* (*P. M. Peterson & N. F. Refulio-Rodriguez 13917*). —D. Habit. —E. Spikelet. —F. Floret.

below, mostly glabrous below, nodes glabrous or with a few hairs, 3 to 5 nodes per culm. Leaf sheaths 1/2 to as long as the internodes, sparsely pilose, the hairs papillose-based up to 2.5 mm, ciliate at the summit; ligules 0.3–0.8 mm, ciliate; blades 3–6(–9) × 0.15–0.3 cm, flat to involute, sparsely pilose, scaberulous above and along margins. Panicles 1.6–8.5 × 0.8–1.2(–3) cm, oblong, spiciform and densely flowered, interrupted below, greenish with a hint of purple, primary branches 0.5–3 cm, ascending, tightly appressed, flowered to base; rachis puberulent and scabrous; pulvini glabrous; pedicels 0.2–0.3 mm. Spikelets 3–4.5 × 1.7–2.2 mm, 4- to 8-flowered, ovate, greenish to stramineous, strongly compressed; disarticulation of entire florets with rachilla attached usually just above the glumes, glumes deciduous; glumes 1–1.6 mm, subequal, ovate, hyaline, membranous, glabrous; apex acute, sometimes mucronate, the mucro less than 0.4 mm; lower glume 1–1.2(–1.4) mm; upper glume 1.3–1.6 mm; lemmas 1.7–2.4 mm, ovate, lateral nerves conspicuous, often green, scaberulous on the keel; apex acute often mucronate, the mucro less than 0.4 mm; paleae 0.8–1.5 mm, hyaline, keels ciliate, the cilia 0.3–0.5 mm, soft and silky; apex obtuse to truncate; stamens 3, anthers 0.2–0.3 mm, reddish brown. Caryopses 0.4–0.6(–0.7) mm, ovoid to ellipsoid, somewhat laterally flattened, striate and reticulate, elliptical-obovate to circular in cross section, reddish brown, sometimes whitish.

Distribution and habitat. Endemic to the coast of Peru and northern Chile; occurs in lomas vegetation in sandy soils and hills in desert habitats; 0–1000 m (Nicora, 1994).

Phenology. Flowering September through February.

Specimens examined. PERU. **Ancash:** Prov. Santa, Lomas de Casma, *R. Ferreyra 8030* (US, USM). **Arequipa:** Prov. Caravelí, Lomas de Atiquipa, *R. Ferreyra 6480* (US, USM); Prov. Mollendo, *C. Vargas C. s.n.* (CUZ). **Ica:** Prov. Nasca, Lomas de Marcona, al S de Nasca, *R. Ferreyra 13376* (US, USM). **La Libertad:** Prov. Trujillo, Cerro Cabezón, *A. Sagástegui A. et al. 10470* (US). **Lambayeque:** Prov. Chiclayo, Cerro Reque, *S. Llatas Q. 335* (CPUN). **Lima:** Prov. Lima, Chorrillos near Lima, *J. F. Macbride 5847* (US). **Moquegua:** Prov. Moquegua, Ilo, Lomas de Mostacilla, *C. Vargas C. 017979* (CUZ). **Tacna:** Prov. Tacna, ca. 21 km SE of Moquegua Tacna at Km 1225 S of Lima, *M. O. Dillon, P. Matekaitis & L. Watanabe 3342* (CPUN, F, US).

19. Eragrostis pilgeri Fedde, Just's Bot. Jahresber. 3: 18. 1908. *Eragrostis andicola* Pilg., Bot. Jahrb. Syst. 37: 377. 1906, nom. illeg. TYPE: Peru. Ancash: Hacienda Cajabamba, entre Samanco y Caraz, 27 May 1903, *A. Weberbauer 3114* (lectotype, designated by Alegría & Granda, Sida 19: 1158. 2001, MOL not seen; isotypes, BAA 999 [fragm.] ex B!, US 2766205 [fragm.]!).

Perennials, caespitose with intravaginal innovations; culms 26–84 cm tall, erect, terete near base, glabrous below the nodes; nodes mostly basal or 1 to 2 above; internodes glabrous. Leaf sheaths 6–16 cm, longer than the lower internode if present, mostly glabrous or with scattered hairs near the summit, the hairs up to 1.3 mm; upper sheaths sometimes with minute, whitish glands along the veins; margins mostly smooth, usually with a large tuft of hairs near the summit, these hairs up to 3 mm; collar visible, yellowish; ligules 0.4–0.7 mm, ciliate, sometimes these hairs extending up to 2.5 mm, these breaking off at maturity; blades (6–)10–22(–26) × 0.1–0.25 (–0.30) cm, flat above the ligule to tightly involute above, apically acuminate, usually densely pilose to villous near base above and below to sparsely pilose to villous near base and glabrous above, the hairs up to 2.5 mm. Panicles 10–20(–38) × 5–15 cm, ovate, open, primary branches mostly 1.5–11 cm, naked near base, the loosely flowered branches spreading 20°–80° from the rachises with spreading secondary branches, 1 to 3 per node; pulvini in the axils of primary and secondary branches glabrous to sparsely villous, the hairs up to 5 mm; pedicels 1.2–7 mm, delicately spreading, sinuous to flexuous, scaberulous and sometimes with a minute, irregularly shaped, whitish gland at apex. Spikelets 3–6.1 × (2–)2.2–4.5 mm, florets 3 to 8, ovate, dark green with small plumbeous spots; rachilla flattened, with hairs more numerous at the base where the floret is attached and sometimes densely ciliate along the margins, the hairs 0.4–1.8 mm; spikelets spreading 30°–45° from the rachilla axis; disarticulation with the glumes first then the lemmas falling individually leaving the paleae on the rachilla; glumes 2–2.8 mm, about equal in length, shorter than the lower lemma, lanceolate to ovate, membranous, keeled, scaberulous along the keel and sometimes with minute, white, raised glands; apex acute to acuminate, often mucronate, the mucro up to 0.5 mm; lemmas 2–3.2 mm, broadly ovate, membranous, lateral veins somewhat obscure, keeled, scaberulous along the keel and near the apex, the veins sometimes with minute, whitish, raised glands; apex acute, often darker than below; paleae 2–3.1 mm, elliptic, membranous, keels usually sometimes with minute, whitish, raised glands; apex truncate to obtuse, sometimes minutely erose; stamens 3, anthers 1.2–2 mm, yellow to purplish at maturity. Caryopses 0.7–0.9 mm, rectangular-prismatic, striate and reticulate, deeply grooved on the ventral surface,

rectangular to triangular in cross section, dark reddish brown.

Distribution and habitat. Endemic to Peru and known only from Ancash and Cajamarca Departments (Peterson et al., 2000); steep rocky slopes, hillsides, and sandy slopes associated with xerophytic plants such as *Agave* L., *Commelina* L., *Lupinus* L., *Puya* Molina, *Vicia* L., *Viguiera* Kunth, and other shrubby Asteraceae; 2200–3200 m.

Phenology. Flowering February through April.

Comments. There has been much confusion with this species from its initial description by Pilger (1906) as *Eragrostis andicola* Pilg., a later homonym (not *E. andicola* R. E. Fr.), and subsequent naming by Fedde (1908) as *E. pilgeri* Fedde. Hitchcock (1927) also replaced *E. andicola* Pilg. with *E. pilgeriana* Hitchc., also a later homonym (not *E. pilgeriana* Dinter ex Pilg). More recently, Alegría Olivera and Granda Paucar (2001) lectotypified *E. pilgeri* and its synonyms and have questioned the validity of *E. ancashensis* P. M. Peterson, Refulio & Tovar. We actually agree with most of their assessment that *E. ancashensis* should not be recognized at the specific level, but we feel the presence of glands on the sheaths, pedicels, glumes, lemmas, and paleae is sufficient to warrant subspecific recognition.

Key to the Subspecies of *Eragrostis pilgeri* in Peru

19a. Glumes, lemmas, and paleae without glands or rarely with a few white glands on midvein of the glume only; pedicels rarely with a minute, irregularly shaped, white gland at apex . . . . . *E. pilgeri* subsp. *pilgeri*

19b. Glumes, lemmas, and paleae with numerous minute, white, raised glands along the veins; pedicels with a minute, irregularly shaped, white gland at apex . . . . . *E. pilgeri* subsp. *ancashensis*

19a. Eragrostis pilgeri Fedde subsp. **pilgeri**. Figures 4M–P; 15D–G.

Eragrostis andicola f. *humilior* Pilg., Bot. Jahrb. Syst. 37: 377. 1906. TYPE: Peru. Ancash: Hacienda Cajabamba, entre Caraz y Samanco, 3700 m, May 1903, *Weberbauer 3036* (lectotype, designated by Alegría & Granda, Sida 19: 1158. 2001, MOL not seen; isotype, MOL not seen).

Eragrostis carazensis Pilg., Bot. Jahrb. Syst. 56(Beibl. 123): 27. 1920. TYPE: Peru. Ancash: Caraz, 2200 2500 m, 12 May 1903, *A. Weberbauer 2999* (lectotype, designated by Alegría & Granda, 2001: 1158, MOL not seen; isotype, BAA 1018 [fragm.] ex B!, US 2891460!).

Upper leaf sheaths usually without minute, white glands along the veins; pedicels rarely with a minute, irregularly shaped, white gland at apex. Spikelets with the rachilla densely or sparsely ciliate, the hairs mostly 0.4–1.8 mm; glumes without or rarely with a few minute, white, raised glands along the keel; lemmas without glands; paleae without glands.

Specimens examined. PERU. **Ancash:** Prov. Corongo, 7 km NW of Bambus, *P. M. Peterson & N. F. Refulio Rodriguez 13915, 13919* (US, USM); Prov. Huaylas, Huascarán Nat. Park, Ausquispuquio, *D. N. Smith, R. Valencia & Buddensick 11959* (HUT); Puente Paria, *Proaño 63* (US). **Cajamarca:** Prov. Cajamarca, Cajamarca, cañada above town, *E. Anderson 661* (US).

19b. Eragrostis pilgeri subsp. **ancashensis** (P. M. Peterson, Refulio & Tovar) P. M. Peterson & Sánchez Vega, stat. et comb. nov. Basionym: *Eragrostis ancashensis* P. M. Peterson, Refulio & Tovar, Sida 19(1): 66. 2000. TYPE: Peru. Ancash: Prov. Recuay, Cordillera Blanca, ca. 20 km E of Raquia on Rt. 02-014, on rd. to Huaraz, 10° 08′55.8″S, 77° 19′48.8″W, 3000 m, 20 Mar. 1997, *P. M. Peterson & N. F. Refulio Rodríguez 13793* (holotype, USM!; isotypes, K!, MO 5156401!, NY!, RSA!, TAES!, UC!, US 3404098!, US 3404099!, WIS!). Figures 4I–L; 20A–K.

Upper leaf sheaths usually with minute, whitish glands along the veins; pedicels with a minute, irregularly shaped, white gland at apex. Spikelets with a rachilla densely ciliate, the hairs 0.8–1.8 mm; glumes with numerous minute, white, raised glands along the keel; lemmas with minute, white, raised glands along the keel and lateral veins; paleae with white glands along the keels.

20. Eragrostis pilosa (L.) P. Beauv. subsp. **pilosa.** Ess. Agrostogr. 71: 162, 175. 1812. Basionym: *Poa pilosa* L., Sp. Pl. 1: 68. 1753. TYPE: "Gramin paniculis elegantissimis, majus, locustis, purpureo-spadiceis, minoribus" in Scheuchzer, Agrostographia: 193, t. 4, f. 3. 1719 (lectotype, designated by Du Puy et al., Fl. Australia: 472. 1993). [EPITYPE: Italy. 9–10 Aug. 1902, *A. Kneucker, Gram. Exsicc. XII, 344* (epitype, designated by H. Scholz in Cafferty et al., Taxon 49: 256. 2000, B!; US 557051!)]. Figures 6E–H; 18D–G.

Annual, caespitose; culms 8–45(–70) cm tall, erect or geniculate spreading below, glabrous or occasionally with a few glandular pits. Leaf sheaths overlapping below, about 1/2 to as long as the internodes above, ciliate at the summit and collar or glabrous, the hairs up to 3 mm; ligules 0.1–0.5 mm, ciliate; blades 2–15(–20) × 0.1–0.25(–0.4) cm, flat, scaberulous

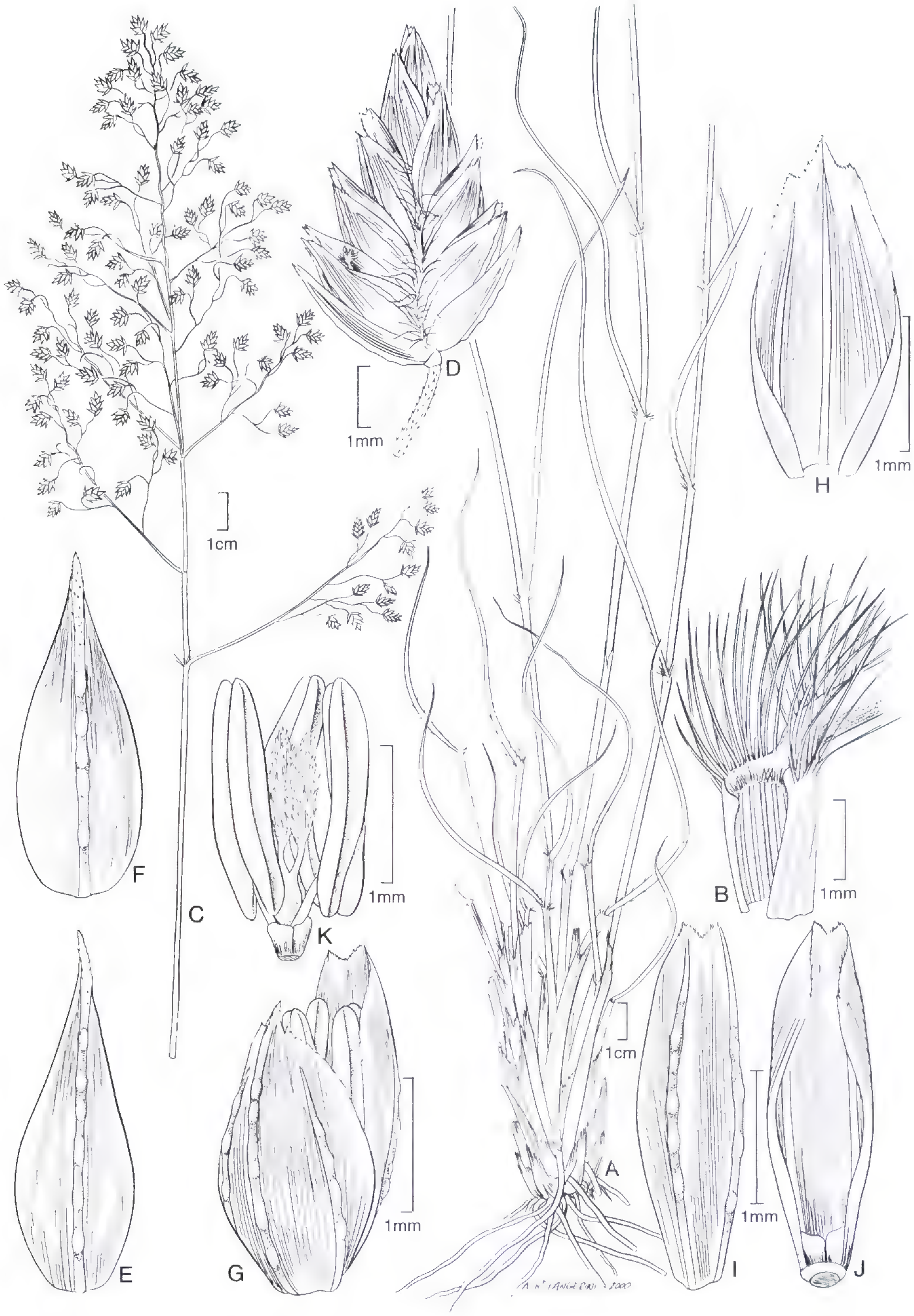

Figure 20. *Eragrostis pilgeri* subsp. *ancashensis* (*P. M. Peterson & N. F. Refulio Rodríguez 13793*). —A. Habit. —B. Ligule. —C. Panicle. —D. Spikelet. —E. Lower glume. —F. Upper glume. —G. Floret. —H. Lemma (ventral view). —I. Palea (dorsal view). —J. Palea enclosing the lodicules. —K. Stamens, pistil, and lodicules.

above and glabrous below with a few hairs near the base. Panicles 4–20(–28) × 2–15(–18) cm, ellipsoid to ovoid, open, diffuse, the ascending, capillary often drooping, primary branches 1–10 cm, spreading 10°–80°(–110°) from the rachises, usually whorled on the lowest two nodes, glabrous to scaberulous; pulvini glabrous to occasionally sparsely ciliate; pedicels 0.8–10 cm, erect to flexuous, appressed to spreading,

scaberulous. Spikelets (2–)3.5–6(–10) × 0.6–1.3 (–1.8) mm, (3 to)5- to 17-flowered, linear-oblong to narrowly ovate, plumbeous; disarticulation acropetal with the glumes first then the lemmas and paleae falling individually, paleae easily deciduous; glumes 0.3–1.2 mm, narrowly ovate to lanceolate, hyaline, keeled, scaberulous along the keel; lower glume 0.3–0.6(–0.8) mm; upper glume 0.7–1.2(–1.4) mm, usually broader than the lower; lemmas 1.2–1.8 mm, ovate-lanceolate, hyaline to membranous, grayish green below and reddish purple near the apex, keeled, scaberulous along the keel near apex, lateral nerves inconspicuous; apex acute; paleae 1–1.6 mm, hyaline to membranous, scaberulous along the keels; apex obtuse; stamens 3, anthers 0.2–0.3 mm, purplish. Caryopses 0.5–1 mm, obovoid to prism-shaped, dorsally flattened, smooth to striate, rectangular in cross section, light brown. $2n$ = 20, 36, 40 (Bir & Sahni, 1988).

Distribution and habitat. Native in Europe, naturalized in North, Central, and South America (excluding Surinam); occurs in disturbed habitats and along forest margins in sandy or gravelly sites; 0–2700 m (Peterson & Boechat, 2001).

Phenology. Flowering September through May.

Specimens examined. PERU. **Cajamarca:** Prov. Cajamarca, Cajamarca, entre Cajamarca y Baños del Inca, *I. Sánchez-Vega & V. Torrel 2373* (CPUN, US). **Huánuco:** Prov. Huánuco, Cerro Huánuco, *O. Velarde 2525* (US). **Lambayeque:** Prov. Lambayeque, Cholocal, ca. a Motupe, *R. Ferreyra 5866* (US, USM); N side of Río Olmos, *J. T. Columbus, J. M. Porter & E. H. Roalson 3151* (RSA, US). **Loreto:** Prov. Maynas, Iquitos, Isla Muy Muy, S of Iquitos, *H. Ellenberg 2882* (US); Padre Isla, entrada a Yarinacocha, *S. McDaniel & M. Rimachi 23051* (AMAZ). **Piura:** Al S de Piura, desvio a Chulucanas, *R. Ferreyra 5918* (US, USM). **Tumbes:** Zarumilla, *H. Ellenberg 1373* (US).

21. Eragrostis soratensis Jedwabn., Bot. Arch. 5(3–4): 213. 1924. TYPE: Bolivia. La Paz: vicinis Sorata, colle Ticacirca, Feb. 1858, *G. Mandon 1331* (lectotype, designated by Hitchcock, Cont. U.S. Natl. Herb. 1927: 343 [without citing a herbarium], W!; isotypes, BAA 1095 ex B!, BM not seen, G not seen, GOET not seen, K!, P!, S not seen, US 1126604!, W!). Figures 5A–D; 21A–C.

Perennial, caespitose with extravaginal innovations; culms 10–40 cm tall, erect to ascending, foliage mostly basal, glabrous, 1 or 2 nodes per culm. Leaf sheaths overlapping, longer than the internodes, glabrous, pilose at the summit and along margins, to pilose on upper half, the hairs up to 2 mm; ligules 0.3–1 mm, ciliate; blades 4–10 × 0.2–0.3 cm, flat to involute above, scabrous on the adaxial surface, sometimes pilose near base and along margins, glabrous abaxially. Panicles 10–20 × 12–19 cm, ovate to pyramidal, open, primary branches 6–12 cm, 1 or 2 per node, naked near base, spreading 25–90° from the rachises; secondary branches composed of loosely overlapping spikelets; pulvini glabrous or with a few hairs; pedicels 1.5–6 mm, erect, spreading, scaberulous. Spikelets 3.4–4.2 × 1–1.6 mm, 4- to 6(to 7)-flowered, plumbeous to purplish green; rachilla somewhat flattened with a few short hairs on the margins, the hairs less than 0.5 mm; disarticulation acropetal, glumes first then lemmas, paleae weakly persistent; glumes 1–1.4 mm, subequal, ovate; lower glume 1–1.2 mm, upper glume 1.2–1.4 mm; lemmas 1.5–1.9 mm, ovate, membranous, lateral nerves inconspicuous, scaberulous along the keel; apex acute, purplish; paleae 1.5–1.9 mm, as long as the lemmas, hyaline; stamens 3, anthers 0.3–0.4 mm, reddish brown. Caryopses 0.6–0.8 mm, obovoid to prism-shaped, striate and reticulate, laterally flattened, ventrally grooved, irregularly rectangular with lateral sides angled, reddish brown.

Distribution and habitat. Native to the Altiplano region of Bolivia and Peru near Lago Titicaca; occurs on rocky slopes and flats in puno vegetation and humid scrub; 2500–4000 m (Renvoize, 1998).

Phenology. Flowering December through May.

Specimens examined. PERU. **Ayacucho:** Prov. Huanca Sancos, 27 km NW of Putajasa & 3 km S of Sacsamarca, *P. M. Peterson, A. Cano, M. LaTorre, A. Ramírez & D. Susanibar 16274* (US, USM). **Cusco:** Prov. Urubamba, start of the Inca Trail at Cusichaca, *B. Peyton & S. Tilney Peyton 224* (US); Ollantaytambo, *A. S. Hitchcock 22517* (US). **Puno:** Prov. Collao, 10 km NW of Pomata on rd. toward Ilave, *P. M. Peterson, N. F. Refulio Rodriguez & F. Salvador Pérez 11625* (US, USM); Juliaca, *H. L. Harlan s.n.* (US).

22. Eragrostis tenuifolia (A. Rich.) Hochst. ex Steud., Syn. Pl. Glumac. 1: 268. 1854. Basionym: *Poa tenuifolia* A. Rich., Tent. Fl. Abyss. 2: 425. 1850 [1851]. TYPE: Ethiopia. In locis incultis Vallium prope Adoam, 18 Sep. 1837, *G. H. W. Schimper 92* (lectotype, designated by Phillips, Fl. Ethiopia 7: 122. 1995, P!; isotypes, GOET 5814 not seen, K!, L not seen, W!, WAG not seen, US 1127147 [fragm.]!). Figures 6I–L; 21D–F.

Perennial, caespitose; culms 25–75 cm tall, erect to geniculate spreading below, glabrous, 2 to 3 nodes per culm. Leaf sheaths overlapping below, 3/4 to as long as the internodes above, glabrous, pilose along the margins and at the summit, the hairs up to 2 mm long;

Figure 21. *Eragrostis soratensis* (*P. M. Peterson, N. F. Refulio-Rodriguez & F. Salvador Perez 14625*). —A. Habit. —B. Spikelet. —C. Rachilla with floret. *Eragrostis tenuifolia* (*P. M. Peterson & O. Tovar 14049*). —D. Habit. —E. Spikelet. —F. Rachilla with floret.

ligules 0.2–0.3 mm, ciliate; blades 3.5–20(–30) × 0.1–0.3 cm, flat to folded or loosely involute, glabrous to scaberulous above and glabrous below. Panicles 5–20 × 3–10(–12) cm, open, ovate to narrowly pyramidal, with a well-developed peduncle up to 18 cm, the relatively few-flowered primary branches 0.5–6 cm, spreading 40°–90° from the rachises, solitary, scaberulous; pulvini ciliate, often reddish, the hairs up to 2 mm; pedicels 2–15 mm, erect, capillary and stiff, scaberulous. Spikelets 6–12(–14) × 1.5–2.2 mm, 6- to 14-flowered, linear, plumbeous; disarticulation acropetal with the glumes first then the lemmas falling, paleae mostly persistent; glumes 0.3–1 mm, very unequal, hyaline, not keeled, glabrous; lower glume 0.3–0.6 mm, subulate to linear-lanceolate; upper glume 0.5–1 mm, lanceolate, usually broader than the lower; lemmas 1.5–2 mm, ovate, membranous, plumbeous, obscurely keeled, lateral nerves obscure, scaberulous near the apex; apex acute to obtuse; paleae 1.3–1.8 mm, membranous, plumbeous, scaberulous along the keels; apex obtuse; stamens 3, anthers 0.2–0.4 mm, red to white in age. Caryopses 0.6–1.1 mm, ovoid, strongly laterally flattened, and curved on the adaxial side, striate and reticulate, with a deep ventral groove, narrowly triangular in cross section, brownish. $2n = 40$ (Pohl & Davidse, 1971; Morton, 1993).

Distribution and habitat. Native in North Africa and Asia, naturalized and rapidly spreading in the New World tropics from Mexico, Guatemala, El Salvador, Honduras, Costa Rica to Argentina, Bolivia, Chile, Colombia, Ecuador, Peru, and Venezuela; along roadsides, city sidewalks, soccer fields, and disturbed open areas; 200–3400 m.

Phenology. Flowering October through June.

Specimens examined. PERU. **Ancash:** Prov. Bolognesi, 8 km E of Raquia & 2 km W of Cajacay on Ruta 02-014, *P. M. Peterson, N. F. Refulio-Rodriguez, A. Cano, M. LaTorre & I. Salinas 17883* (US, USM). **Apurímac:** Prov. Aymaráes, 21 km NW of Chalhuanca, *P. M. Peterson & N. F. Refulio Rodriguez 16518* (US, USM). **Cajamarca:** Prov. Cutervo, near Sócoto, *I. Sánchez Vega 4559* (CPUN, HAO); 13 km W of Cutervo on rd. toward Sócoto, *P. M. Peterson & N. F. Refulio-Rodriguez 15012* (US, USM); Prov. San Ignacio, 21 km W of Huahuaya & 13 km E of Tamborapa, *P. M. Peterson & N. F. Refulio-Rodriguez 15110* (US, USM). **Cusco:** Prov. Urubamba, Museo sitio Machupichu, *C. Vargas C. 017424* (CUZ); 69 km de Cusco, entre Tancacc y Ollanta, *P. Vuñez V. & E. Bengoa 8731* (CUZ, US). **Junín:** Prov. Tarma, 1 km up rd. to Hac. Maraynioc out of Palca, *P. M. Peterson & O. Tovar 14049* (US, USM). **Piura:** Prov. Ayabaca, Añarte, ruta a Tondopa, *S. Llatas Q. 2151* (CPUN); Prov. Huancabamba, 10 km N of Sondor & 3 km S of Huancabamba, *P. M. Peterson & N. F. Refulio-Rodriguez 15168* (US, USM).

23. Eragrostis weberbaueri Pilg., Bot. Jahrb. Syst. 37: 375. 1906. TYPE: Peru. Ancash: Pampa Roman entre Samanco y Caraz, 2300 m, 29 May 1903, *A. Weberbauer 3189* (holotype, B not seen; isotype, BAA 1110 [fragm.] ex B!, US 2767411!). Figures 3E–H; 19D–F.

Perennial or biennial, caespitose; culms 14–35 cm tall, erect, ascending to geniculate below, 3 nodes per culm, nodes silky pilose, the hairs up to 3 mm long, internodes with scattered hairs to glabrous. Leaf sheaths 1/2–2/3 as long as the internodes, striate, pilose, densely pilose near summit, the hairs colorless or white; ligules 0.7–1 mm, ciliate, the hairs less than 1 mm long; blades 6–9 × 0.1–0.17 cm, flat at base, folded or involute above, usually densely silky pilose above and below. Panicles 3–8(–11) × 0.5–1 cm, spiciform, narrowly oblong, clavate, sometimes interrupted below, dark green to plumbeous, rachis densely pilose, the hairs not rigid, primary branches (0.7–)1–1.7 mm, short, closely appressed, hairy; pedicels 0.2–0.3 mm. Spikelets 4–6 × 1.2–1.5 mm, 6- to 11-flowered, linear-ovate to oblong, tightly appressed, plumbeous; disarticulation acropetal, with glumes first then lemmas, paleae and rachilla persistent; glumes 1–1.8 mm, unequal, membranous; lower glume (0.8–)1–1.4 mm; upper glume 1.3–1.8 mm; lemmas 2–2.5 mm, ovate, membranous to hyaline, scabrous near apex; apex acute; paleae 1.5–1.7 mm, shorter than lemma and narrower, hyaline. Stamens 3, anthers ca. 0.4–0.5 mm, purple. Caryopses 0.6–0.7 mm, ellipsoid, faintly striate and reticulate, circular to ovate in cross section, reddish orange.

Distribution and habitat. Native to the coast of northern Chile and Peru; occurs in lomas vegetation, and western slopes of the Andes where taxa from higher elevations appear perennial and those from immediate coast appear to be annual or biennial; 0–3500 m.

Phenology. Flowering March and April.

Specimens examined. PERU. **Ancash:** Prov. Bolognesi, 8 km E of Raquia & 2 km W of Cajacay on Ruta 02-104, *P. M. Peterson, N. F. Refulio-Rodriguez, A. Cano, M. LaTorre & I. Salinas 178776* (US, USM); Prov. Corongo, 7 km NW of Yupan on rd. to Bambus, *P. M. Peterson & N. F. Refulio Rodriguez 13917* (US, USM); River Valley Colca Fortaleza, *E. Anderson 454* (US). **Arequipa:** Prov. Arequipa, Cerros de Yura (Baños), *C. Vargas C. 7978* (CUZ); S slope of Nevado Chachani, *J. T. Columbus, J. M. Porter & E. H. Roalson 3535* (RSA, US); 21 km N of Yura on hwy. toward Pathuasí, *P. M. Peterson & N. F. Refulio Rodriguez 18239* (US, USM); Prov. Camaná, entre Camaná y Arequipa, Km 161–162 Panamericana, *R. Ferreyra 2557* (US, USM); 23 km NE of Camaná, *J. T. Columbus, J. M. Porter & E. H. Roalson 3523* (RSA, US); Prov. Caravelí, 13 km S of Cahuacho on rd. toward Caravelí, *P. M. Peterson, M. LaTorre, A. Ramírez & D. Susanibar 16398* (US, USM). **Ica:** 32 km E of Nasca on rd. toward Puquio, *P. M. Peterson & N. F. Refulio-Rodriguez 16425* (US, USM). **Lima:** Matucana, *F. Macbride & Featherstone 303* (US).

Excluded Name

Poa patula Kunth, Nov. Gen. Sp. 1: 158–159. 1815 [1816]. *Eragrostis patula* (Kunth) Steud., Nom. Bot. (ed. 2) 1: 564. 1840. *Megastachya patula* (Kunth) Roem. & Schult., Syst. Veg. 2: 585. 1817. TYPE: Ecuador. Apr.–May, *F. Humboldt & A. Bonpland s.n.* (holotype, P!; isotype, US 2766206 [fragm.] ex P!, P photo at US!).

Comments. The status of this species and its use are questionable. Recent use of this name in Bolivia (Renvoize, 1998), Argentina (Nicora, 1998), and Ecuador (Jørgensen & León-Yánez, 1999) places this as a synonym of *Eragrostis tenuifolia*. However, after studying the type at P and fragment at US, we think that this taxon is not a synonym of *E. tenuifolia*. We cannot find any existing specimens from Peru that resemble the type. Therefore, we are not including this name in this treatment.

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Appendix 1. List of names and synonyms. Accepted names are presented in boldface and synonyms are italicized.

Cynodon amabilis (L.) Raspail [= **E. tenella**]
Cynodon ciliaris (L.) Raspail [**E. ciliaris** var. **ciliaris**]
Diandrochloa glomerata (Walter) Burkart [**E. japonica**]

Diandrochloa japonica (Thunb.) A. N. Henry [= **E. japonica**]

Eragrostis alba J. Presl [= **E. mexicana** (Hornem.) Link subsp. **mexicana**]

Eragrostis amabilis (L.) Nees [= **E. tenella**]

Eragrostis amabilis var. *plumosa* (Retz.) E. G. Camus & A. Camus [= **E. tenella**]

Eragrostis ancashensis P. M. Peterson, Refulio & Tovar [= **E. pilgeri** subsp. **ancashensis**]

Eragrostis andicola Pilg. [= **E. pilgeri**]

Eragrostis andicola R. E. Fr.

Eragrostis andicola f. *humilior* Pilg. [= **E. pilgeri** subsp. **pilgeri**]

Eragrostis andicola var. *robustior* Pilg. [= **E. pilgeri** subsp. **ancashensis**]

Eragrostis atrovirens (Desf.) Trin. ex Steud.

Eragrostis attenuata Hitchc.

Eragrostis bahiensis var. *boliviensis* Henrard [= **E. lurida** subsp. **lurida**]

Eragrostis buchtienii Hack. [= **E. pastoensis**]

Eragrostis carazensis Pilg. [= **E. pilgeri** subsp. **pilgeri**]

Eragrostis cilianensis (All.) F. T. Hubb. [= **E. cilianensis**]

Eragrostis cilianensis (All.) Vignolo ex Janch.

Eragrostis ciliaris (L.) Nees [= **E. ciliaris** var. **ciliaris**]

Eragrostis ciliaris (L.) R. Br. var. **ciliaris**

Eragrostis ciliaris var. *patens* Chapm. ex Beal [= **E. tenella**]

Eragrostis contracta Pilg. [= **E. lurida** subsp. **contracta**]

Eragrostis contristata Nees & Meyen [= **E. lurida** subsp. **lurida**]

Eragrostis curvula (Schrad.) Nees

Eragrostis deserticola Phil. [= **E. peruviana**]

Eragrostis diffusa Buckley [= **E. pectinacea** var. **pectinacea**]

Eragrostis glomerata (Walter) L. H. Dewey [= **E. japonica**]

Eragrostis hypnoides (Lam.) Britton, Sterns & Poggenb.

Eragrostis japonica (Thunb.) Trin.

Eragrostis lehmannii Pilg. [= **E. pastoensis**]

Eragrostis limbata E. Fourn. [= **E. mexicana** subsp. **mexicana**]

Eragrostis lugens Nees

Eragrostis lurida J. Presl

Eragrostis lurida subsp. **contracta** (Pilg.) P. M. Peterson & Sánchez Vega

Eragrostis lurida J. Presl subsp. **lurida**

Eragrostis magna Hitchc.

Eragrostis maypurensis (Kunth) Steud.

Eragrostis megastachya var. *cilianensis* (All.) Asch. & Graebn. [= **E. cilianensis**]

Eragrostis mexicana (Hornem.) Link

Eragrostis mexicana (Hornem.) Link subsp. **mexicana**

Eragrostis mexicana subsp. **virescens** (J. Presl) S. D. Koch & Sánchez Vega

Eragrostis montufari (Kunth) Steud. [= **E. pastoensis**]

Eragrostis multiflora var. *cilianensis* (All.) Maire [= **E. cilianensis**]

Eragrostis neomexicana Vasey ex L. H. Dewey [= **E. mexicana** subsp. **mexicana**]

Eragrostis nigricans (Kunth) Steud.

Eragrostis nigricans var. *tristis* (Jedwabn.) Pilg. [= **E. nigricans**]

Eragrostis olmedoi (Kunth) Steud. [= **E. pastoensis**]

Eragrostis pastoensis (Kunth) Trin.

Eragrostis pectinacea (Michx.) Nees var. **pectinacea**

Eragrostis pectinacea (Michx.) Steud. [= **E. pectinacea** var. **pectinacea**]

Eragrostis peruviana (Jacq.) Trin.

Eragrostis peruviana var. *brachythyrsa* Pilg. [= **E. peruviana**]

Eragrostis pilgeri Fedde

Eragrostis pilgeri subsp. **ancashensis** (P. M. Peterson, Refulio & Tovar) P. M. Peterson & Sánchez Vega

Eragrostis pilgeri Fedde subsp. **pilgeri**

Eragrostis pilgeriana Hitchc. [= **E. andicola**]

Eragrostis pilosa (L.) P. Beauv. subsp. **pilosa**

Eragrostis pilosa var. *lugens* (Nees) Griseb. [= **E. lugens**]

Eragrostis plumosa (Retz.) Link [= **E. tenella**]

Eragrostis purshii var. *diffusa* (Buckley) Vasey [= **E. pectinacea** var. **pectinacea**]

Eragrostis rahmeri Phil. [= **E. nigricans**]

Eragrostis scabra Phil. [= **E. nigricans**]

Eragrostis setifolia (Benth.) Steud. [= **E. pastoensis**]

Eragrostis soratensis Jedwabn.

Eragrostis subatra Jedwabn. [= **E. nigricans**]

Eragrostis tenax (Kunth) Steud [= **E. pastoensis**]

Eragrostis tenella (L.) P. Beauv. ex Roem. & Schult.

Eragrostis tenella var. *japonica* (Thunb.) Roem. & Schult. [= **E. japonica**]

Eragrostis tenella var. *plumosa* (Retz.) Stapf [= **E. tenella**]

Eragrostis tenuifolia (A. Rich.) Hochst. ex Steud.

Eragrostis tristis Jedwabn. [= **E. nigricans**]

Eragrostis virescens J. Presl [= **E. mexicana** subsp. **virescens**]

Eragrostis virescens var. *trachyphylla* Hack. [= **E. pastoensis**]

Eragrostis weberbaueri Pilg.

Erosion cilianense (All.) Lunell [= **E. cilianensis**]

Erosion hypnoides (Lam.) Lunell [= **E. hypnoides**]

Koeleria multiflora Regel & Herter [= **E. peruviana**]

Megastachya amabilis (L.) P. Beauv. [= **E. tenella**]

Megastachya ciliaris (L.) P. Beauv. [= **E. ciliaris** var. **ciliaris**]

Megastachya glomerata (Walter) Schult. [= **E. japonica**]

Megastachya hypnoides (Lam.) P. Beauv. [= **E. hypnoides**]

Megastachya maypurensis (Kunth) Roem. & Schult. [= **E. maypurensis**]

Megastachya montufari (Kunth) Roem. & Schult. [= **E. pastoensis**]

Megastachya nigricans (Kunth) Roem. & Schult. [= **E. nigricans**]

Megastachya olmedoi (Kunth) Roem. & Schult. [= **E. pastoensis**]

Megastachya pastoensis (Kunth) Roem. & Schult. [= **E. pastoensis**]

Megastachya tenax (Kunth) Roem. & Schult. [= **E. pastoensis**]

Megastachya tenella (L.) Bojer [= **E. tenella**]

Neeragrostis hypnoides (Lam.) Bush [= **E. hypnoides**]

Poa amabilis L. [= **E. tenella**]

Poa atrovirens Desf. [= **E. atrovirens**]

Poa cilianensis All. [= **E. cilianensis**]

Poa ciliaris L. [= **E. ciliaris** var. **ciliaris**]

Poa glomerata Walter [= **E. japonica**]

Poa hypnoides Lam. [= **E. hypnoides**]

Poa japonica Thunb. [= **E. japonica**]

Poa lurida (J. Presl) Kunth [= **E. lurida**]

Poa maypurensis Kunth [= **E. maypurensis**]

Poa mexicana Hornem. [= **E. mexicana**]

Poa montufari Kunth [= **E. pastoensis**]

Poa nigricans Kunth [= **E. nigricans**]
Poa olmedoi Kunth [= **E. pastoensis**]
Poa pastoensis Kunth [= **E. pastoensis**]
Poa pectinacea Michx. [= **E. pectinacea** var. **pectinacea**]
Poa peruviana Jacq. [= **E. peruviana**]
Poa pilosa L. [= **E. pilosa** subsp. **pilosa**]
Poa plumosa Retz. [= **E. tenella**]
Poa setifolia Benth. [= **E. pastoensis**]
Poa tenax Kunth [= **E. pastoensis**]
Poa tenella L. [= **E. tenella**]
Poa tenuifolia A. Rich. [= **E. tenuifolia**]
Roshevitzia japonica (Thunb.) Tzvelev [= **E. japonica**]
Sporobolus scaber Phil. [= **E. attenuata**]

A REVIEW OF THE GENUS *DISTICTIS* (BIGNONIACEAE)[1,2]

Amy Pool[3]

Abstract

Distictis Mart. ex Meisn. is a genus of 11 species in the tribe Bignonieae. All the species are lianas and can be recognized by their ribbed hexangular branchlets without interpetiolar glandular fields; bifoliolate leaves with a trifid (or occasionally additionally branched) terminal tendril; terminal inflorescence; usually glandular, campanulate, more or less truncate calyces; more or less straight, tubular-infundibular or tubular-campanulate corollas that are white, purple, orange, or red; usually elliptic, non-echinate capsules with both ends pointed, and often with both valves convex; and usually bialate seeds, with the body brown and ridged and often papillose or pubescent, and the wings usually well demarcated from the seed body and membranous-hyaline. Relationships with similar genera are discussed, and a key to the species of *Distictis*, species descriptions, and species distribution maps are provided. The relationships of the species are also discussed, and a new combination, *D. frutescens* (DC.) A. Pool, is proposed. A neotype is designated for *D. granulosa* Bureau & K. Schum., and lectotypes are designated for *Bignonia rigescens* Jacq., *Pithecoctenium buccinatorium* DC., *P. buccinatorium* var. *subinclusum* DC., *P. buccinatorium* var. *exsertum* DC., *P. cinereum* DC., and *P. uleanum* Kraenzl. *Pithecoctenium stipulare* Mart. ex DC. and *D. stipularis* Mart. ex Glaz. are presented as new synonyms of *D. frutescens*, *P. cinereum* var. *parviflorum* Hochr. is presented as a new synonym of *D. laxiflora* (DC.) Greenm., and *P. scabriusculum* var. *macranthum* Bureau & K. Schum. is presented as a new synonym of *D. scabriuscula* (Mart. ex DC.) A. H. Gentry.

Key words: Bignoniaceae, *Distictis*, Neotropical, Pithecocteniinae.

Distictis Mart. ex Meisn. is a Neotropical genus of 11 species in the tribe Bignonieae, which can generally be recognized by the combination of a number of vegetative, floral, and fruiting characters. As are most members of the tribe Bignonieae, *Distictis* is a genus of lianas that has compound leaves with the terminal leaflet modified into a tendril, bilocular ovaries, and fruits that dehisce parallel to the septum (Gentry, 1980). *Distictis* has branchlets hexangular and ribbed, the ribs often detaching with age, no interpetiolar glandular fields, and often foliaceous pseudostipules. The leaves are bifoliolate with a terminal 3(to 5)-fid tendril.

The inflorescence is a terminal raceme or panicle, with or without conspicuous bracteoles, usually with few to several flowers (numerous in *Distictis pulverulenta* (Sandwith) A. H. Gentry). The calyces are campanulate, pubescent, more or less apically truncate and denticulate, and usually have distinct glandular fields. Most species of *Distictis* have flowers with a weakly zygomorphic corolla that is tubular-infundibular, or tubular-campanulate from a cylindric base, and externally pubescent, with the tube internally glabrous (excluding trichomes at level of insertion of stamens), all four stamens and the stigma included in the corolla, and a pubescent ovary sitting on a large pulvinate nectar disc. Four species (*D. frutescens* (DC.) A. Pool, *D. granulosa* Bureau & K. Schum., *D. laxiflora* (DC.) Greenm., *D. scabriuscula* (Mart. ex DC.) A. H. Gentry) have corollas with purple lobes and usually yellow throats, and four (*D. gnaphalantha* (A. Rich.) Greenm., *D. lactiflora* (Vahl) DC., *D. occidentalis* A. H. Gentry, *D. steyermarkii* A. H. Gentry) have corollas white or white with yellow throats. Two species (*D. buccinatoria* (DC.) A. H. Gentry and *D. staminea* (Lam.) A. H. Gentry) have red or orange corollas and differ from the other species in the genus in having subexserted to exserted stamens and stigma. *Distictis staminea* also differs from the other species in having a markedly bilabiate corolla, and both *D. staminea* and *D. lactiflora* are unusual in the genus in having ovaries that are strongly lepidote with few, if any, trichomes present. *Distictis pulverulenta* differs from the other species of *Distictis* in having dark red- or brown-purple corollas and calyces and the inner surface of the corolla tube densely pubescent and lepidote.

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The capsules of *Distictis* are somewhat more variable than the flowers. They are elliptic in shape and more or less pointed at each end. Both valves are convex or slightly compressed (*D. buccinatoria*) to strongly compressed (*D. scabriuscula*), except for *D. pulverulenta*, which has one valve convex and the other concave, resulting in a curved fruit. The valves are externally pubescent in the South American species but glabrous or nearly so in those from Central America and the West Indies. The seeds are bialate with the body brown and ridged and either puberulent or papillose in most of the species. The wings are generally well demarcated from the seed body, large, membranous and hyaline, except in *D. occidentalis* and *D. pulverulenta*. The seeds of *D. occidentalis* have a more or less corky body and greatly reduced and coriaceous wings, and the seeds of *D. pulverulenta* have the wings poorly demarcated from the body and hyaline only at their apices. Mature capsules and seeds were not seen for *D. frutescens*, *D. gnaphalantha*, and *D. staminea*.

All the South American species of *Distictis* (except *D. pulverulenta*) have simple unbranched racemes, corollas with glandular fields at the base of the lobes, and dendritic trichomes present on petioles, petiolules, and/or leaflets and usually on branchlets and inflorescence also.

By and large, the genus holds together very well. Species that vary from the norm do so in a manner that fits well-established patterns in the Bignoniaceae (as discussed in the Reproductive Biology section of this paper) and are closely paired to another species that fits the typical mold. *Distictis pulverulenta* is the one exception. *Distictis pulverulenta* was originally described in *Distictella* Kuntze by Sandwith (1938) and transferred by Gentry (1976) to *Distictis* based on its hexangular, ribbed branchlets and straight, chartaceous to subcoriaceous, purple corollas, all of which contrast with *Distictella*, which has terete branchlets and curved, coriaceous, white corollas. However, *Distictis pulverulenta* is an oddity in the genus *Distictis* based on several vegetative, floral, and fruiting characteristics that are common in *Distictella* but otherwise unknown in *Distictis*: the many-flowered, compoundly branched panicle, the pubescent and lepidote inner surface of the corolla tube, and the curved fruits. In addition, the pubescence of the undersurface of the leaves is nearly identical to that of an unpublished species of *Distictella* and the seeds are very similar to those of *Distictella elongata* (Vahl) Urb., with wings that are poorly demarcated from the seed body, yellow and hyaline at the tips, and brown and opaque at the base, and with the same irregular ridges and papillae as found on the body. The generic placement of *Distictis pulverulenta* is uncertain, but, pending further study, it is here retained in *Distictis* following Gentry (1976, 1982b).

History

Meisner established the genus *Distictis* in 1840, crediting Martius with the genus name. He made no new combinations but referred to Vahl's (1794) description and plate of *Bignonia lactiflora* Vahl (= *D. lactiflora*) and Jacquin's (1797) description and plate of *B. rigescens* Jacq. (= *D. lactiflora*). Meisner's (1840) important key characteristics for *Distictis* included seeds more than uniseriate, with membranous wings, calyx simple and truncate, and capsule non-echinate.

A. P. de Candolle (1845) made the combinations in *Distictis* alluded to by Meisner; added *D. arthrerion* (Mart.) DC. (= *Arrabidaea arthrerion* (Mart.) Bureau ex K. Schum.; Schumann, 1894); published the new species *Pithecoctenium frutescens* DC. and *P. stipulare* Mart. ex DC. (= *D. frutescens*), *P. buccinatorium* DC., including two varieties (= *D. buccinatoria*), *P. laxiflorum* DC. and *P. cinereum* DC. (= *D. laxiflora*), and *P. scabriusculum* Mart. ex DC. (= *D. scabriuscula*); and left *Bignonia staminea* Lam. (= *D. staminea*) in *Bignonia* with a "?", perhaps due to the poor quality of the Burman (1756) plate.

Miers (1863) transferred *Pithecoctenium buccinatorium* to his new genus, *Phaedranthus* Miers, publishing also *Phaedranthus lindleyanus* Miers, *P. exsertus* Miers, *P. cinerascens* nom. nud. (all synonymous with *Distictis buccinatoria*), and *P. extatus* Miers (= *Arrabidaea elegans* (Vell.) A. H. Gentry; Gentry, 1975). The important characteristics Miers (1863) used to separate *Phaedranthus* from *Pithecoctenium* Mart. ex Meisn. were large foliaceous pseudostipules, straight, bright purple corolla, and exserted stamens and style.

Bureau (1864) misapplied the name *Distictis* to what is now referred to as *Distictella* and published the new genus, *Macrodiscus* Bureau (= *Distictis*), and combination *M. rigescens* (Jacq.) Bureau (= *D. lactiflora*). Bureau (1864) distinguished *Macrodiscus* (= *Distictis*) from *Distictis* (= *Distictella*) primarily on fruit characters, the capsule of *Macrodiscus* (= *Distictis*) being glabrous (vs. pubescent) and uncurved (vs. curved with one convex and one concave valve), with a furrow in place of a raised midrib, free replum ends extending beyond the tip of the septum (vs. attached), seeds glabrous (vs. pubescent), with relatively shorter wings, and seed scars on the septum punctiform (vs. linear).

Bentham (1876) included both *Macrodiscus* and *Distictis* sensu Bureau in his concept of the genus *Distictis*, in which he identified three groups: (1)

Distictis arthrerion (= *Arrabidaea arthrerion*), (2) *Distictis mansoana* (DC.) Bureau (= *Distictella mansoana* (DC.) Urb.) and *Distictis elongata* (Vahl) Bureau ex K. Schum. (= *Distictella elongata* (Vahl) Urb.), and (3) *Macrodiscus* group: *Distictis lactiflora*, *Distictis rigescens* (Jacq.) DC. (= *Distictis lactiflora*), and *Bignonia gnaphalantha* A. Rich. (= *Distictis gnaphalantha*), noting the curved versus uncurved capsules in distinguishing between groups 2 and 3.

Baillon (1891), Schumann (1894), and Bureau and Schumann (1896) recognized and carried on Bureau's original misapplication of *Distictis* and *Macrodiscus*. Schumann (1894) separated *Distictis* (as *Macrodiscus*) from *Pithecoctenium*, *Distictella* (as *Distictis*), and other related genera based on floral characters: the membranous to subcoriaceous corolla not curving at a right angle versus coriaceous corolla bent at nearly a right angle. In *Flora Brasiliensis*, Bureau and Schumann (1896) retained *P. scabriusculum* (= *Distictis scabriuscula*) and *P. stipulare* (= *D. frutescens*) in *Pithecoctenium* with notes that they might pertain to another genus, and published the new species, *Distictis granulosa*. Schumann (Bureau & Schumann, 1896) acknowledged the different use of the name *Distictis* by de Candolle versus Bureau and indicated that the latter use was employed in the *Flora Brasiliensis*.

Greenman (1898) made the combination *Distictis gnaphalantha* (as suggested by Bentham, 1876), transferred *Pithecoctenium laxiflorum* to *Distictis* (= *D. laxiflora*) based on its fruiting characteristics, and transferred *P. cinereum* to *Distictis* (= *D. laxiflora*) based on its similarities to *D. laxiflora* and *D. gnaphalantha*.

Urban (1908) published the new genus *Wunschmannia* Urb., based on *Bignonia staminea* (= *Distictis staminea*), and observed its affinity with *Macrodiscus* (= *Distictis*) and, in particular, *M. lactiflorus* (Vahl) Bureau ex K. Schum. (= *D. lactiflora*), noting that it differed most importantly in its clearly bilabiate corolla and exserted stamens (Urban, 1908). Urban (1916a) recognized Bureau's misapplication of the genus name *Distictis* and transferred many of the names published in *Distictis* post Bureau to *Distictella*.

Sprague and Sandwith (1932b) discussed the close relationship that exists between *Pithecoctenium stipulare* (= *Distictis frutescens*), *P. scabriusculum* (= *D. scabriuscula*), and *D. granulosa*, which they transferred to *Pithecoctenium*, and enumerated the differences between *P. stipulare* (= *D. frutescens*) and *P. granulosum* (Bureau & K. Schum.) Sprague & Sandwith (= *D. granulosa*), which provided helpful insights in the preparation of this paper.

Pichon (1945) established the genus *Anomoctenium* Pichon based on *Pithecoctenium stipulare* (= *Distictis frutescens*), separating it from *Pithecoctenium* based on its large, foliaceous pseudostipules and the pollen, which he found to be esulcate and finely reticulate, but with gaps in the reticulation.

Sandwith (1962) selected *Distictis lactiflora* as the lectotype of the genus *Distictis*. Sandwith (1965) expanded the concept of *Anomoctenium* to include *Pithecoctenium granulosum* (= *D. granulosa*) and *P. scabriusculum* (= *D. scabriuscula*). He examined the pollen of *A. stipulare* (Mart. ex DC.) Pichon (= *D. frutescens*) but did not find the areas void of reticulation described by both Urban (1916b) and Pichon (1945) and, in comparing it to the pollen of *P. echinatum* (Jacq.) Baill. (= *P. crucigerum* (L.) A. H. Gentry) and *Distictella mansoana*, found all three to be "essentially similar, spherical, unfurrowed and continuously reticulate over the exine" (Sandwith, 1965: 412). Sandwith (1965) described his expanded version of *Anomoctenium* as having dendritic trichomes on all parts, branchlets more or less hexangular, with detachable ribs, purplish corollas with glandular fields externally at the base of the lobes, capsules pubescent with both valves convex and the apex beaked-acuminate, and seeds in two rows, more narrowly transverse-oblong than those of *Pithecoctenium* with a brown, rough and ridged body and hyaline-membranous wings of darker color than those of *Pithecoctenium*. He also compared *Anomoctenium* to *Pithecoctenium*, *Distictella*, and *Amphilophium* Kunth and suggested that *Anomoctenium* should join these three genera in the subtribe Pithecocteniinae of Melchior (1927).

Gentry (1973) placed *Phaedranthus* in synonymy with *Distictis* based on the floral and vegetative similarity of *P. buccinatorius* (DC.) Miers and *D. laxiflora* and, supported by their history of successful hybridization, made the new combination, *D. buccinatoria*.

Gentry (1974a) published a synopsis of the genus *Distictis*, recognizing nine species. In addition to accepting the previously published combinations *D. lactiflora*, *D. gnaphalantha*, *D. laxiflora*, and *D. buccinatoria*, he placed *Wunschmannia* and *Anomoctenium* in synonymy, made three species-level transfers into *Distictis*, and published one new species. These are discussed below. Gentry's (1974a) synonymy, both at the genus and the species levels, has been followed in this paper.

Urban (1908) separated *Wunschmannia* from *Distictis* (as *Macrodiscus*) based on its bilabiate corolla and exserted stamens. Gentry (1974a) noted that the similarities between *Wunschmannia* and *Distictis*, hexangular, ribbed branchlets, leaf form, terminal inflorescence, subfoliaceous pseudostipules, truncate, campanulate calyces, sometimes with glandular fields,

and strongly pubescent corollas, outweighed their differences, particularly as his expanded concept of *Distictis* included *D. buccinatoria*, a species with subexserted to exserted stamens. He therefore placed *Wunschmannia* in synonymy with *Distictis* and transferred *W. staminea* (Lam.) Urb. to *Distictis*.

When Sandwith (1965) revised *Anomoctenium* to include *Pithecoctenium granulosum* and *P. scabriusculum*, he compared his new concept of *Anomoctenium* with *Pithecoctenium* and *Distictella* but failed to include *Distictis* in his discussion. When Gentry (1974a) contrasted *Distictis* with Sandwith's interpretation of *Anomoctenium*, he found that the two differed only in *Anomoctenium* possessing dendritic trichomes and corollas with glandular fields. In addition, Gentry (1974a) found the two genera to have a number of characters in common: usually trifid (to several-branched) tendrils, foliaceous pseudostipules, terminal inflorescences, often with leaf-like bracteoles, and very similar corollas, calyces, and pollen. He therefore placed *Anomoctenium* in synonymy with *Distictis*, transferred *A. stipulare* (= *D. frutescens*) and *P. scabriusculum* to *Distictis*, and recognized *A. granulosum* (Bureau & K. Schum.) Sandwith as *D. granulosa*. A new species belonging to the *Anomoctenium* group was published as *D. steyermarkii*.

Gentry (1976) discussed the similarities and differences between *Distictis* and *Distictella* as first recognized by Bureau (1864), modified by Schumann (1894), and, finally, in light of new species described in or transferred to both genera post Bureau and Schumann. Bureau (1864) was the first to recognize *Distictis* (as *Macrodiscus*) and *Distictella* (as *Distictis*) as two closely related genera, distinguished primarily by fruiting characters as described above. Bentham (1876) treated both within the same genus, but as separate groups, one with curved fruits and the other without. Schumann (1894) separated *Distictis* (as *Macrodiscus*) from *Pithecoctenium*, *Distictella* (as *Distictis*), and other related genera based on floral characters: the membranous to subcoriaceous corolla not curving at a right angle versus coriaceous corolla bent at nearly a right angle. De Candolle (1845) described the branchlets of *Distictis* as subangulate and striate, while Sprague and Sandwith (1932b), Schumann (1894, as *Distictis*), and Melchior (1927) described the branchlets of *Distictella* as terete without striation, or without angles with detachable ribs. Gentry (1976) outlined the generic differences described by Bureau (1864) and Schumann (1894) and found that when species described or transferred to *Distictis* post-Bureau (1864) were examined, the fruit differences did not correspond well with the vegetative and floral characteristics. For example, *Distictis granulosa*, *D. scabriuscula*, and *D. steyermarkii* all have pubescent capsule and seeds (characters of *Distictella*) but have the flowers and branchlets of *Distictis*. Likewise, *Distictella monophylla* Sandwith and *D. obovata* Sandwith have flowers and twigs of *Distictella* but uncurved fruits and glabrous seeds, characteristics previously associated with *Distictis*. Gentry concluded that, "In general the characters of the fruit elucidated by Bureau lack taxonomic significance at the generic level" (Gentry, 1976: 78), and transferred *Distictella pulverulenta* Sandwith (which has a pubescent, curved fruit with seeds with poorly demarcated wings) to *Distictis* based on its hexangular, ribbed stems and straight, chartaceous to subcoriaceous and purple corollas.

In 1992, Gentry published another new species in the genus *Distictis*, *D. occidentalis*. It is a member of the *Anomoctenium* group and, in most earlier collections, had been identified as *D. granulosa*.

Anatomy

Santos (1995) studied 31 genera of Bignonieae and divided them into four groups based on the type of cambial variant present. Two species of *Distictis*, *D. granulosa* and *D. occidentalis*, were included in her study. A third species, *D. pulverulenta*, is discussed, but the MO sheet of the voucher cited (*G. dos Santos et al. 279*) is clearly *D. granulosa*. *Distictis* fits into her "group 2," which is characterized by stems with multiples of four phloem wedges in transverse section. The genera generally thought to be the most closely related to *Distictis*, *Distictella* and *Pithecoctenium* (Sandwith, 1965; Gentry, 1976; Gentry & Tomb, 1979), belong to Santos' "group 4," which has stems with discontinuous phloem wedges intersected with xylem and parenchyma (Santos, 1995). *Amphilophium*, another genus that is considered closely related by both Sandwith (1965) and Gentry (1974a), is included with *Distictis*, and a large number of other genera, in Santos' "group 2."

Palynology

Gentry and Tomb (1979) included *Distictis buccinatoria* and *D. occidentalis* (reported as *D. granulosa*) in their SEM survey of pollen types in the Bignoniaceae. From this, they concluded that *Distictis* has inaperturate, coarse-reticulate pollen (Gentry & Tomb, 1979). This is supported by the earlier light microscopic studies of Urban (1916b) and Sandwith (1965). Unfortunately, Urban (1916b) did not cite species or vouchers examined. However, it appears that species of *Distictis* as currently circumscribed were included in four of the genera he surveyed. He included *D. lactiflora* and *D. gnaphalantha* in his

discussion of *Distictis* and described the pollen of this genus as spherical, esulcate (= inaperturate) with large spaces between the meshwork of the reticulation (= coarse-reticulate). *Wunschmannia* (= *D. staminea*) was described as having spherical and esulcate pollen with a large-mesh reticulation. *Distictis laxiflorum* (as *Pithecoctenium cinereum*) was included in his discussion of *Pithecoctenium*, and *Pithecoctenium* was described as having spherical, esulcate pollen with moderately large-mesh reticulation, except for *P. stipulare* (= *D. frutescens*), which was said to have moderate-sized gaps in the network of the exine. Pichon (1945) found these same gaps, but Sandwith (1965) found the pollen of this species to be spherical, unfurrowed, and continuously reticulate. Urban (1916b) described the pollen of *Phaedranthus* (= *D. buccinatoria*) as spherical and esulcate, with large reticulation and the walls of the mesh having outgrowths. These same outgrowths were described for *Distictella broadwayana* Urb. (= *Distictella racemosa* (Bureau & K. Schum.) Urb.), and Sandwith (1965: 412) suggested that Urban "was confusing exaggerated bulging sinuosities of the walls with outgrowths."

It seems probable that all members of the genus *Distictis* as currently circumscribed do have similar pollen: spherical, inaperturate with coarse reticulation. Gentry and Tomb (1979) found this pollen type in a number of other genera, which they suggested could be divided into two natural groups. The one including *Distictis* is characterized by having thick-textured white to purple or red flowers, trifid to multitrifid tendrils, and multiseriate ovules and seeds. The other genera in the group are *Pithecoctenium* and *Distictella*, genera that Sandwith (1965) and Gentry (1976) had previously suggested as closely related. *Amphilophium*, another genus suggested as closely related (Sandwith, 1965; Gentry, 1974a), had pollen of a different type, stephanocolpate (zonocolpate) with coarse reticulation (Gentry & Tomb, 1979), a pollen type shared with *Glaziovia* Benth. & Hook. f., and *Haplolophium* Cham. (including *Urbanolophium* Melch.), all of which are characterized by a frilly outer calyx margin, unique in the family (Gentry & Tomb, 1979).

Generic Placement Within the Tribe Bignonieae

Melchior (1927) established the subtribe Pithecoctenioinae, which included *Pithecoctenium*, *Distictella*, *Neves-armondia* K. Schum. (= *Pithecoctenium*), *Urbanolophium* (= *Haplolophium*, following Gentry, 1992), *Haplolophium*, *Glaziovia*, and *Amphilophium*. The subtribe was described as having bifoliolate or trifoliolate leaves, tendrils usually trifid (rarely bifid), or additionally forked, and often with disk-like tips; the calyx thickly coriaceous and densely tomentose, with or without defined lobes, the corolla coriaceous, and, except in *Amphilophium*, densely pubescent, often curved at a right angle and sometimes more or less bilabiate; and the capsule oblong-elliptic, flattened, the surface smooth or rough to echinate, and the seed with a membranous wing. *Distictis* was not included in Melchior's concept of the subtribe, perhaps due to its less coriaceous corolla and shape of the capsule valves; the species that would have been considered to be in *Distictis* at that time, *D. lactiflora*, *D. gnaphalantha*, and *D. laxiflora*, all have convex capsule valves that are not at all flattened. However, in other respects *Distictis* does fit the description of Pithecocteniinae. It also shares a number of additional similar features with individual genera within the subtribe. All of the genera have seeds with at least the seed body irregularly ridged or papillose, and most have at least some species with pubescent seed bodies. *Distictis*, *Pithecoctenium*, and *Distictella* all have a similar pollen type (Gentry & Tomb, 1979), and *Distictis* and *Pithecoctenium* both have hexangular, ribbed branchlets, seeds with the wings (in most species) well demarcated from the seed body, and, at least sometimes, many-branched tendrils. *Pithecoctenium* and *Distictella* both differ from *Distictis* in having corollas that are strongly curved and always white. *Distictis*, *Amphilophium*, and most species of *Haplolophium* have branchlets that are acutely hexangular and share the same type of cambial variant (Santos, 1995). In addition, many of the species in all three genera have at least some branched trichomes. Both *Amphilophium* and *Haplolophium* differ from *Distictis* in pollen type (Gentry & Tomb, 1979) and in their calyces, which have an expanded and frilly margin, and *Amphilophium* also differs in having pseudo-cleistogamous corollas. The close relationship of *Distictis* with members of the Pithecocteniinae was suggested by Sandwith (1965; as *Anomoctenium*), Gentry (1974a, 1976), and Gentry and Tomb (1979).

Distribution

Distictis is a Neotropical genus, known from central and southern Mexico, throughout the West Indies, and northern South America to Peru, Bolivia, and Brazil. Two species, *D. buccinatoria* (Fig. 1A) and *D. laxiflora* (Fig. 2A), are found from central to southern Mexico, in rocky areas or deciduous forests above 1000 m, and *D. laxiflora* is also known from northwestern Nicaragua in similar habitats, above 1000 m. Three species are found in the West Indies in dry forest below 1000 m: *D. staminea* (Fig. 1A),

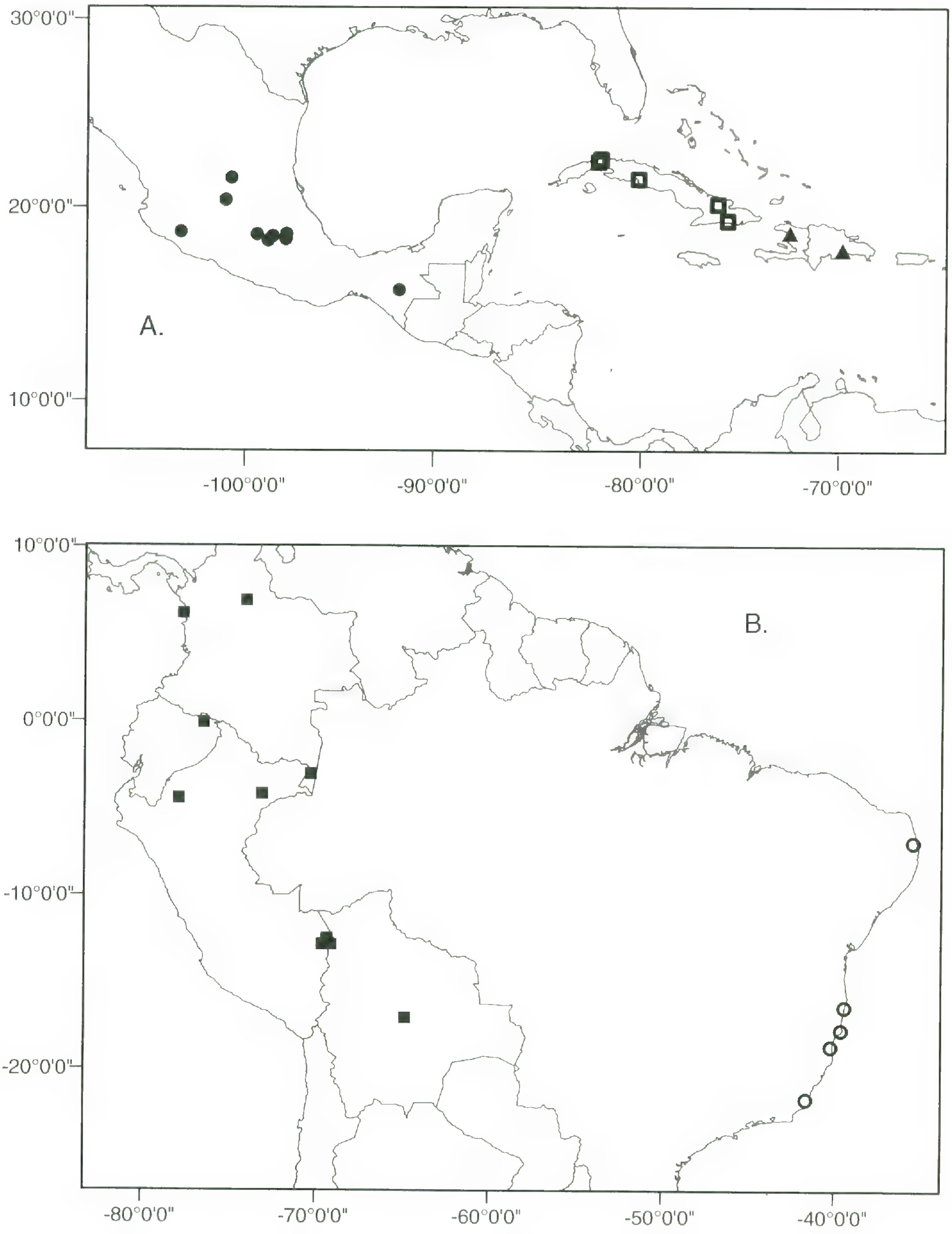

Figure 1. Distribution of selected species of *Distictis*. —A. *Distictis buccinatoria* (DC.) A. H. Gentry (●), *D. gnaphalantha* (A. Rich.) Greenm. (■), and *D. staminea* (Lam.) A. H. Gentry (▲). —B. *Distictis frutescens* (DC.) A. Pool (○) and *D. occidentalis* A. H. Gentry (■).

endemic to Hispaniola; *D. gnaphalantha* (Fig. 1A), endemic to Cuba; and *D. lactiflora* (Fig. 2A), widespread in Cuba, Haiti, Dominican Republic, Puerto Rico, St. Croix, and St. Thomas. The remaining species are South American. One species, *D. steyermarkii* (Fig. 2A), is found along the northern border of Colombia with Venezuela and on the Atlantic coast of Venezuela in very dry, deciduous forests between sea level and 1400 m. Two species, *D. frutescens* (Fig. 1B) and *D. scabriuscula* (Fig. 2B), are

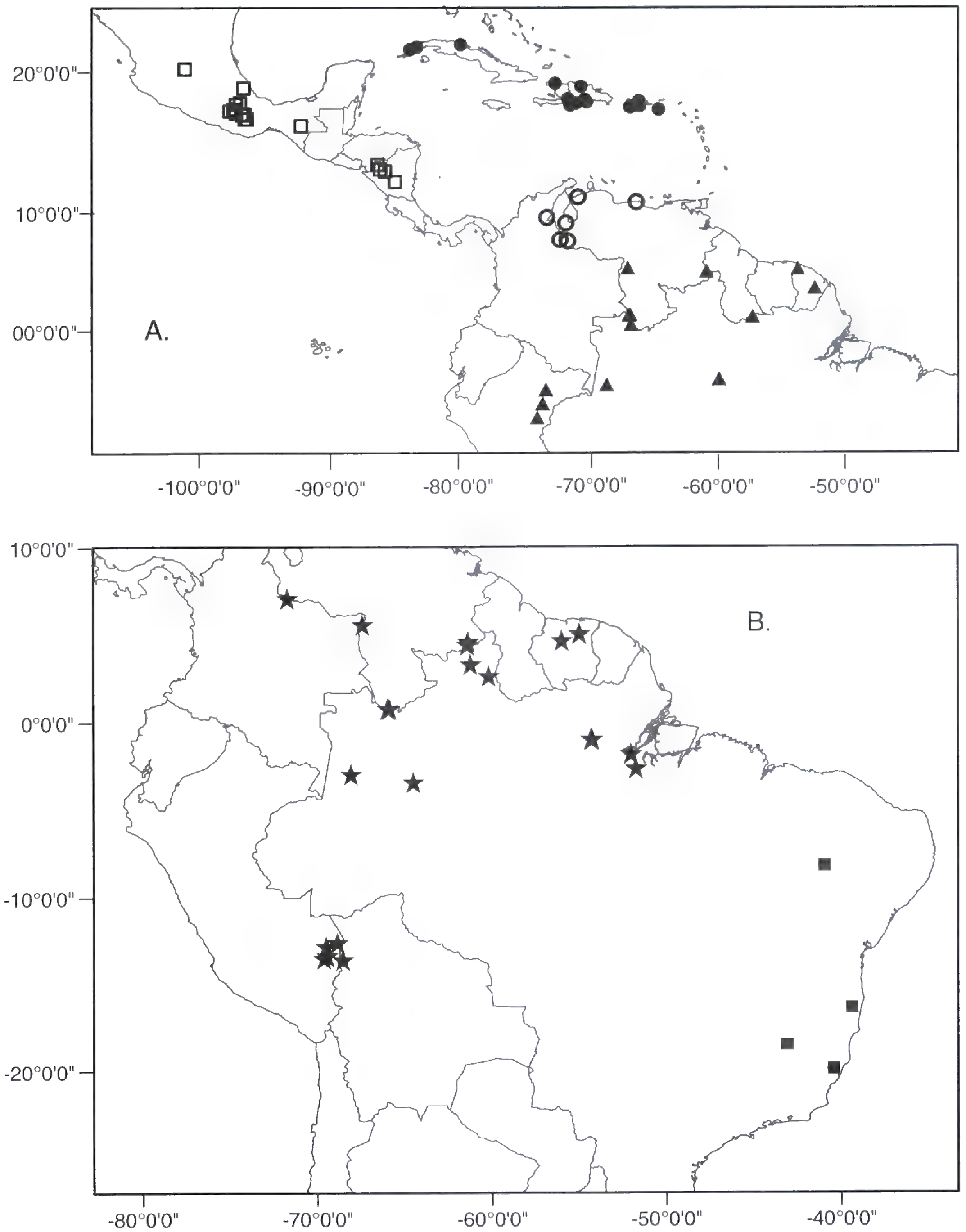

Figure 2. Distribution of selected species of *Distictis*. —A. *Distictis lactiflora* (Vahl) DC. (●), *D. laxiflora* (DC.) Greenm. (□), *D. pulverulenta* (Sandwith) A. H. Gentry (▲), and *D. steyermarkii* A. H. Gentry (○). —B. *Distictis granulosa* Bureau & K. Schum. (★) and *D. scabriuscula* (Mart. ex DC.) A. H. Gentry (■).

endemic to eastern Brazil, both probably growing in wet forest, *D. frutescens* below 100 m and *D. scabriuscula* below 1000 m. *Distictis occidentalis* (Fig. 1B) is the westernmost species of *Distictis* in South America and is found from Colombia (Chocó and Santander) south to Peru (Madre de Dios) and Bolivia (Santa Cruz), usually in seasonally inundated forests or along rivers between 100 and 300 m. *Distictis granulosa* (Fig. 2B) is found in the central Amazon and Guianas as well as southeastern Peru

(Madre de Dios and Puno) in moist to wet forests between 30 and 1200 m. The distribution ranges of *D. granulosa* and *D. occidentalis* overlap at Tambopata, Peru, but *D. occidentalis* is found in swampy areas around lake margins, while *D. granulosa* is found in sandy upland forests (Gentry, 1992). *Distictis pulverulenta* (Fig. 2A) is found in a northern Amazonian arc, stretching from the Guianas west and south through southern Venezuela, southern Colombia, northwestern Brazil, and northeastern Peru, in non-inundated forests on white sand (Gentry, 1979a) between 120 and 900 m.

Reproductive Biology

Gentry (1974b, 1980) recognized 10 major morphological floral types within the Bignoniaceae, each with its own correlating pollination agent(s). Most species of *Distictis* seem to be adapted toward bee pollination (Gentry, 1990). They have coriaceous calyces that are generally pubescent and glandular, corollas that have white to purple lobes and a yellow throat and are moderately thick in texture and pubescent with the base of the tube narrow and more coriaceous, included stamens and style, and a large nectary disc. Several species have additional glandular fields on the outer surface of the corolla tube, directly below the lobes. Flowers of *Distictis* are somewhat intermediate between the *Anemopaegma*-type and *Pithecoctenium*-type flowers described by Gentry (1974b). The *Anemopaegma* type is the most common in the Bignoniaceae. It is characterized by thin membranous corollas with an open mouth and included anthers. There is considerable variation within this group. The flowers may be yellow, white, blue, or purple, often with a contrastingly colored throat. They may be externally pubescent, glabrous, or lepidote. All are conspicuous and produce large quantities of nectar. They are pollinated by large- to medium-sized bees but may also suffer heavily from nectar robbery (Gentry, 1974b). *Pithecoctenium*-type flowers appear to be designed to thwart nectar robbers. They have very thick protective calyces, and corollas that are externally densely pubescent (above the calyx), are very thick (especially at the base), and have an internal sturdy ridge of thickened (perhaps glandular) trichomes at the level of the insertion of stamens that nearly closes the tube (Gentry, 1974b). In addition to the above characteristics, flowers in this group are generally strongly bent or curved below the middle, are white or cream colored, and produce abundant nectar (Gentry, 1974b). Other genera in this group include *Distictella* and *Ceratophytum* Pittier (Gentry, 1974b). The fairly thick, pubescent calyces and corollas of most species of *Distictis* probably help to reduce the number of successful nectar robbers (Gentry, 1974b), and the glands on the calyces (and in some cases corollas) probably attract ants, which provide further protection against nectary robbers and help protect the developing ovary against herbivory (Elias & Gelband, 1975).

Four of the nine species of bee-pollinated *Distictis* have white corollas, while five have corolla lobes that are shades of purple. Of the four white-flowered *Distictis*, *D. lactiflora* and *D. gnaphalantha* are endemic to the West Indies; Gentry (1990) noted that white flowers are common in the Greater Antilles. Gentry (1990) also noted that in *Tabebuia* Gomes ex DC., all of the "core-habitat" species have yellow or magenta flowers, while the habitat specialists have white flowers. *Distictis* seems to follow the same pattern. All of the purple-flowered *Distictis* in South America are found in wet or moist forest, but one of the white-flowered species, *D. occidentalis*, is commonly found in seasonally inundated forests (Gentry, 1992), while the other, *D. steyermarkii*, is found in very dry, deciduous forests.

Distictis staminea and *D. buccinatoria* appear to be hummingbird pollinated (Gentry, 1990). They both have coriaceous calyces, corollas that are orange to red, and subexserted to exserted stamens, and they fit more or less into Gentry's (1974b) *Martinella* type. The *Martinella* type is characterized as having bright red-orange or deep red-violet flowers that produce abundant nectar and are odorless. The corolla is generally rather thick and glabrous outside with an open mouth and a long tube, and the stamens are often exserted or subexserted. The calyx is usually large and membranous and is loose around the base of the corolla tube. Both *D. staminea* and *D. buccinatoria* differ from this standard type in having coriaceous calyces and externally pubescent corolla tubes. *Distictis staminea* also varies from the standard in the corolla being chartaceous and not very long.

Change in pollinators within a genus is rare in the Bignoniaceae. Gentry (1990) reported that only 18% of the Neotropical genera of Bignoniaceae have more than one type of pollen vector and that "such changes appear to be associated with the major genetic reorganization that accompanies generic differentiation" (Gentry, 1990: 125). In fact, both hummingbird-pollinated species of *Distictis* have been treated in separate monotypic genera, *Phaedranthus* and *Wunschmannia*. However, each of the hummingbird-pollinated species of *Distictis* is closely paired with a bee-pollinated species.

Nonfruiting specimens of *Distictis buccinatoria* and *D. laxiflora* are difficult to distinguish, the only reliable characters being the exsertion of the stamens,

color of the corolla, and a tendency for the flowers of *D. buccinatoria* to be larger. The horticultural hybrid that exists between the two further supports their inclusion in the same genus.

Vegetatively, *Distictis staminea* is very similar to *D. lactiflora*, the two differing only in the color and symmetry of the corolla, exsertion of the stamens, and foliar pubescence. It is interesting to note that one of the two hummingbird-pollinated species, *D. staminea*, is endemic to Cuba. Gentry (1990) reflected that a switch in pollinators is common in the Greater Antilles.

Gentry (1974b) recognized five different basic patterns in flowering phenology in the Bignoniaceae. Patterns in phenology are often difficult to recognize from a herbarium study, particularly when the geographic range of the species is large and the number of collections relatively few; however, a few patterns are worth noting. In this herbarium study, the months in which individual species were seen to flower varied from two to nine. The one with the greatest range in flowering time (December–July, September) was *Distictis lactiflora*, a species found throughout much of the West Indies. Gentry (1990) noted that steady-state flowering, lacking seasonality, was a common phenomenon in the Greater Antilles. *Distictis gnaphalantha*, endemic to Cuba, appears to have two flowering seasons per year, once during the main dry season (February–May) and again during a secondary dip in rainfall (July). Few specimens of *D. staminea*, endemic to Hispaniola, have been seen by either myself or A. H. Gentry; it is known to flower in January (the driest month), March (moderately wet), and May (the wettest month). *Distictis laxiflora*, from Mexico and Nicaragua, flowers March to August, and *D. buccinatoria*, from Mexico, flowers April to August; both of these flowering times are at the end of the dry season through the peak of the wet season. *Distictis steyermarkii*, from Venezuela and Colombia, flowers in February and March (dry season) and again in August, October, and November (toward the end of wet season). *Distictis frutescens*, from the Atlantic coast of Brazil, flowers August to November (end of dry to wet season) and March (wet season), while *D. scabriuscula*, endemic to central-eastern Brazil, flowers February and March (wet season). Two of the three basically Amazonian species flower probably once per year, *D. granulosa* from July to March (relatively dry to wet season) and *D. occidentalis* from April to July (dry season), with one collection seen in flower in October. The third species, *D. pulverulenta*, seems to have two flowering seasons per year, May to July (wet season) and November through December (wet season).

Most species of *Distictis* appear to be wind dispersed. *Distictis buccinatoria*, *D. granulosa*, *D. lactiflora*, *D. laxiflora*, *D. scabriuscula*, and *D. steyermarkii* have seeds that are clearly bialate; the wings are well demarcated from the seed body, often large, and membranous-hyaline. *Distictis pulverulenta* also has large membranous wings, but they are opaque except at the apices and are not well demarcated from the seed body. *Distictis occidentalis* has seeds with wings greatly reduced and coriaceous and the body somewhat corky, suggesting that it is water dispersed and correlating well with its usual habitat of seasonally inundated forests or growth along rivers. Shifts from wind dispersal to water dispersal within genera of Bignoniaceae are not uncommon (Gentry, 1983), and Gentry (1973: 227) stated "each water-dispersed species is most closely related to and presumably derived from a different wind-dispersed one." For *D. gnaphalantha* and *D. staminea*, I have seen neither seeds nor descriptions of seeds. I have not seen the seeds of *D. frutescens*, but Gomes (1952) described the seeds as small winged and dark brown, suggesting that they might be similar to those of *D. occidentalis*. *Distictis frutescens* is frequently described as growing along rivers.

Economic Uses

Distictis buccinatoria (Mexican Blood Trumpet), *D. laxiflora* (Vanilla Distictis or Vanilla Trumpet Vine), and their horticultural hybrid, *Distictis* cv. 'Rivers' (marketed as "Distictis riversii"), are cultivated throughout the tropical and subtropical areas of the world as ornamentals for their beautiful flowers (Menninger et al., 1970; Bailey et al., 1976). *Distictis laxiflora* has sometimes been confused in the horticultural trade with *D. lactiflora* (Menninger et al., 1970), but following Bailey et al. (1976) plants grown under the latter name are probably *D. laxiflora*.

Materials and Methods

All specimens examined by Gentry and as part of this study have been incorporated into the Missouri Botanical Garden database-management system TROPICOS, <http://mobot.mobot.org/W3T/Search/vast.html>, which now contains label information for all *Distictis* specimens housed at MO in addition to those received on loan from AAU, B, BM, BR, C, CAS, CGE, CM, F, FTG, G, GH, GOET, L, M, MICH, NY, S, TEX, U, UPS, US, and W. Specimens determined by this author were selected from this database to generate the index to numbered exsiccatae (Appendix 1) and the distribution maps, the latter with the assistance of Duan Bills. Dots on the maps (Figs. 1, 2) represent a subset of the specimens examined by this author, including specimens with geographic coordinates provided on collection labels and

a set of specimens for which the author estimated the geographic coordinates, selected to illustrate the full geographic range for each species.

Wet and dry season designations were made based on Brawer (1991) and the Internet site: <http://www.travel.dk.com/wdr/>.

The term furfuraceous-dendritic trichome is used to describe a very small-branched trichome that Gentry sometimes referred to as thick-stellate (Gentry, 1992). Furfuraceous-dendritic trichomes differ from stellate trichomes in that the arms of the trichome do not radiate from one point, and from other dendritic trichomes in that there are no distinct internodes between the arms of the trichome.

Taxonomic Treatment

Distictis Mart. ex Meisn., Pl. Vasc. Gen. 1: 300, 2: 208. 1840. TYPE: *Distictis lactiflora* (Vahl) DC. [based on *Bignonia lactiflora* Vahl] (lectotype, designated by Sandwith, 1962: 454).

Phaedranthus Miers, Proc. Roy. Hort. Soc. London 3: 182. 1863, nom. cons. TYPE: *Phaedranthus lindleyanus* Miers.

Macrodiscus Bureau, Monogr. Bignon. 46, t. 11. 1864. TYPE: *Macrodiscus rigescens* (Jacq.) Bureau [based on *Bignonia rigescens* Jacq.].

Wunschmannia Urb., Symb. Antill. 5: 194. 1908. TYPE: *Wunschmannia staminea* (Lam.) Urb. [based on *Bignonia staminea* Lam.].

Anomoctenium Pichon, Bull. Soc. Bot. France 92: 226. 1946. TYPE: *Anomoctenium stipulare* (Mart. ex DC.) Pichon [based on *Pithecoctenium stipulare* Mart. ex DC.].

Lianas, indumentum of simple and/or dendritic trichomes; branchlets hexangular and ribbed, the ribs sometimes detaching with age, without interpetiolar glandular fields; pseudostipules often foliaceous or subfoliaceous, or less often small and bract-like to absent, persistent to caducous. Leaves bifoliolate, frequently with a tendril; tendril terminal, apically 3(rarely 5)-fid, sometimes with tips disk-like. Inflorescence terminal (or terminal and lateral), raceme or panicle; bracts and bracteoles narrow, conspicuous to inconspicuous, persistent or caducous, flowers few to several (numerous in *Distictis pulverulenta*); calyx campanulate, usually with glandular zones externally below the margin, apex ± truncate and denticulate; corolla white to cream, lilac to mauve, magenta to deep red-purple or dark yellow to orange or red, weakly zygomorphic or rarely strongly bilabiate (*D. staminea*), tubular-infundibular or tubular-campanulate from cylindric base, tube straight to slightly arching, externally pubescent, internally usually glabrous except for level at which stamens are inserted (tomentose in *D. pulverulenta*); lobes wide, imbricate, pubescent, sometimes with glandular fields externally at base; stamens 4, inserted at same level in corolla tube, generally included or at least the longer pair subexserted to exserted (*D. staminea* and *D. buccinatoria*), anthers glabrous, dithecal, the thecae straight, divaricate or subparallel, glabrous, staminode present, small, inserted at same level as stamens; disc annular-pulvinate; ovary pubescent (or conspicuously lepidote, *D. lactiflora* and *D. staminea*), ovules several-seriate in each locule. Fruit a capsule, elliptic or elliptic-oblong, generally pointed at both ends, woody, non-echinate, both valves convex or one valve convex and the other concave resulting in a curved fruit (*D. pulverulenta*) or both slightly compressed (*D. buccinatoria*) to strongly compressed (*D. scabriuscula*), septum parallel to valves, without raised edges; seeds in 2 rows, body brown with irregular ridges, usually somewhat papillose or puberulose, bialate, wings usually well demarcated from the body, large, membranous and hyaline, rarely poorly demarcated (*D. pulverulenta*) or poorly demarcated, greatly reduced, coriaceous and opaque (*D. occidentalis*).

Phaedranthus Miers is placed in synonymy of *Distictis* based on the many vegetative and floral similarities that its single species shares with *D. laxiflora*, from which it is difficult to distinguish without fruit, the similarity of its pollen type to that of *D. occidentalis* (Gentry & Tomb, 1979), the similarities of its fruit and seeds to that of many species of *Distictis*, and its successful horticultural hybridization with *D. laxiflora* (Gentry, 1973). The placement of *Phaedranthus* in synonymy of *Distictis* follows Gentry (1973, 1974a). *Phaedranthus* is conserved against the rejected name *Sererea* Raf. (McNeill et al., 2006) following Sandwith's (1955) proposal. *Sererea* was published in 1838 (Rafinesque, 1838) and would have priority over *Distictis* (Meisner, 1840) if the two were considered synonymous. When Rafinesque (1838) published his new monotypic genus, *Sererea*, he provided a brief genus description, followed by the new combination, *S. heterophylla* Raf., based on *Bignonia heterophylla* Willd. (published as a superfluous name for *B. kerere* Aubl.). Below this species name, he cited the original Aublet (1775) plate of *B. kerere* Aubl. (as *B. "cherere"*) and plate 1301 in Lindley (1830). A short species description was also included. The Lindley (1830) plate that Rafinesque (1838) cited, while labeled *B. "cherere"* Aubl., is not this species and is very different from the Aublet (1775) plate and description. The Lindley plate depicts a plant with large foliaceous pseudostipules, flowers in a panicle, the corolla red above the base, and slightly exserted stamens. The Aublet plate illustrates a plant with scale-like pseudostipules, flowers in a small raceme, the corolla (in description)

yellow, and the stamens and stigma well included. Rafinesque's description of *Sererea* is clearly based on Lindley's plate and description. Miers (1863) published the new genus *Phaedranthus*, selecting *P. lindleyanus* Miers as the type of the genus. The latter was published without description but validated by referring to the Lindley (1830) publication, and the type was apparently *W. Lambert s.n.*, "in hort. Soc. Hort. Cult. (anno 1834)." Sandwith (1955) believed *Sererea* and *Phaedranthus* to be synonyms because the descriptions of both were based on the plate and description of *B. "cherere"* sensu Lindley, and he believed that Rafinesque's choice of type species *S. heterophylla* based on *B. heterophylla* Willd. "auct. *B. cherere* Aubl." should be treated as an error, taken by inference from Lindley. In other words, Sandwith believed that Lindley's plate should be treated as the type of *Sererea*. However, following Article 10.4 of the *International Code of Botanical Nomenclature* (McNeill et al., 2006), a specimen or illustration that is not the type of a name of an included species can be made the type of the name of a genus only through the act of conservation. As this was not done for *Sererea*, the type of the genus remains the only species included by Rafinesque, *S. heterophylla* (nom. superfl. for *B. kerere*), a species now treated in *Mansoa* as *M. kerere* (Aubl.) A. H. Gentry (Gentry, 1979b), and *Sererea* therefore is a synonym of *Mansoa* and not synonymous with *Distictis* (including *Phaedranthus*).

Macrodiscus Bureau is placed in synonymy of *Distictis* based on the synonymy of its type, *M. rigescens* (Jacq.) Bureau, with the lectotype of *Distictis*, *D. lactiflora* (Vahl) DC.

The monotypic genus *Wunschmannia* Urb. is placed in synonymy of *Distictis* based on its hexangular, strongly ribbed branchlets, subfoliaceous pseudostipules, bifoliate leaves with a terminal, trifid tendril, terminal inflorescence, denticulate calyx, and strongly pubescent corolla, all characteristics common in *Distictis*. Urban's (1916b) description of the pollen of *W. staminea* also closely matches Gentry and Tomb's (1979) description of pollen of *D. buccinatoria* and *D. occidentalis*. The distinguishing characteristics of *W. staminea*, its strongly bilabiate, red corolla and long-exserted stamens, are probably associated with a switch of pollinators from bee to hummingbird. Red corollas and exserted stamens are also present in *D. buccinatoria*.

Anomoctenium Pichon was described by Pichon (1945) with one species, *A. stipulare* (Mart. ex DC.) Pichon, and expanded by Sandwith (1965) to include *A. granulosum* and *Pithecoctenium scabriusculum*. *Anomoctenium* species differs from other *Distictis* only in the presence of dendritic trichomes on most parts and the glandular fields found externally at the base of the corolla lobes. Species of *Anomoctenium* are similar to most species of *Distictis* in having strongly ribbed, hexangular branchlets; bifoliate leaves with a terminal, trifid tendril; denticulate to truncate calyx with external glandular fields; pubescent corolla; elliptic, non-echinate capsules with both valves convex (not seen for *A. stipulare*); and seeds with the wings well demarcated and membranous-hyaline (not seen for *A. stipulare*). Sandwith's (1965) description of the pollen of *A. stipulare* also closely matches Gentry and Tomb's (1979) description of pollen of *D. buccinatoria* and *D. occidentalis*. The similarities of *Anomoctenium* and *Distictis* outweigh the differences, and I agree with Gentry (1974a) in placing *Anomoctenium* in synonymy with *Distictis*.

Key to Species of *Distictis*

1. Dendritic trichomes present on petioles, petiolules, and/or leaflets, and usually also on branchlets and inflorescence (often mixed with simple trichomes in *D. steyermarkii*); linear glandular fields present externally at base of corolla lobes; inflorescence a raceme.
   2. Simple trichomes present at least on adaxial surface of leaflets, longest dendritic trichomes (rarely 0.3–)0.7–2 mm long, furfuraceous-dendritic trichomes absent from vegetative parts; leaflets with bases usually clearly cordate (rarely subcordate); corolla lobes white . . . . . 11. *D. steyermarkii*
   2′. Simple trichomes absent, longest dendritic trichomes rarely to 0.3 mm long, furfuraceous-dendritic trichomes present on young branchlets, petioles, and petiolules; leaflet bases various, sometimes subcordate; corolla lobes white (*D. occidentalis*) or various shades of purple.
      3. Pseudostipules 6–20 × 7–20 mm, persistent; leaflet tertiary venation reticulate, not parallel or ladder-like; bracteoles 13–22 × 2–8 mm; calyx glandular fields short and broad; corolla lobes lilac to mauve . . . . . 2. *D. frutescens*
      3′. Pseudostipules absent, or 2–4 × 0.5–4 mm and caducous; leaflet tertiary venation generally parallel and ladder-like; bracteoles 1.5–12 × 0.5–2 mm; calyx glandular fields narrow and oblong or, if short and broad, then corolla lobes white.
         4. Corolla lobes white; seeds with wings reduced, coriaceous and opaque; calyx glandular fields short and broad . . . . . 7. *D. occidentalis*
         4′. Corolla lobes purple, lavender, or purple-red; seeds with well-developed wings, membranous and hyaline; calyx glandular fields narrow and oblong.
            5. Leaflets subcoriaceous to coriaceous with midrib and lateral veins impressed on the adaxial surface; bracteoles usually persistent, 1.5–2 × 1 mm; calyx with strongly appressed

furfuraceous-dendritic trichomes; throughout northern South America, including Peru and Bolivia, and in Brazil, in areas bordering the Amazon and its tributaries . . . . . 4. *D. granulosa*

5′. Leaflets membranous to chartaceous, midrib and lateral veins usually flat on adaxial surface; bracteoles caducous, 6–11 × 0.5–1 mm; calyx with at least some trichomes with well-developed rachis and these trichomes spreading; restricted to central-eastern Brazil . . . . . 9. *D. scabriuscula*

1′. Dendritic trichomes absent; corollas without glandular fields; inflorescence a panicle or raceme.

6. Corolla strongly bilabiate, lower lip reflexed with 3 equal lobes, upper lip erect and bilobate; longer pair of stamens far exserted; ovary densely lepidote with scattered trichomes at base; endemic to Hispaniola . . . . . 10. *D. staminea*

6′. Corolla weakly zygomorphic; longer pair of stamens included, or if exserted, then ovary densely sericeous; Mexico, Central America, West Indies, and South America.

7. Corolla tube internally tomentose and lepidote; corolla lobes dark red- or brown-purple; capsule curved, with one valve convex and the other concave; seeds with wings poorly demarcated from seed body; leaflets with abaxial surface drying greenish gray, usually densely furfuraceous-tomentose with the main veins glabrescent, occasionally the surface glabrescent, with large glands in conspicuous clusters in lower lateral vein axils; northern South America . . . . . 8. *D. pulverulenta*

7′. Corolla tube internally glabrous, excluding trichomes at level of insertion of stamens; corolla lobes red, orange-red, white, lavender, or magenta; capsules not curved, both surfaces convex to slightly compressed; seeds with wings well demarcated from seed body (not known for *D. gnaphalantha*); leaflets with abaxial surface drying yellow-green to dark green, golden green, or brownish green, glabrous or, if pubescent, pilose, villous, or puberulent, with trichomes overall or restricted to veins or vein axils, large glands scattered, often more dense in lower lateral veins axils, but not forming conspicuous clusters; Mexico, Central America, and West Indies.

8. Corolla lobes red or orange-red, longer pair of stamens subexserted to exserted; capsules oblong-elliptic, 13–15 × 4–5.5 cm, apex acute with tip rounded and retuse . . . . . 1. *D. buccinatoria*

8′. Corolla lobes white, white with pink or lavender flush, magenta, or lavender, stamens well included; capsule elliptic or elliptic-fusiform, 3–10.5 × 1.5–3.5 cm, apex acute to acuminate.

9. Leaflets subcoriaceous to coriaceous with veins conspicuously prominent adaxially, glabrous abaxially; ovaries densely lepidote, or lepidote and puberulent, style glabrous . . . . 5. *D. lactiflora*

9′. Leaflets membranous with veins flat or impressed adaxially, pubescent abaxially, at least on main veins at base; ovaries densely sericeous, style pubescent at least at base.

10. Corolla white, 3.5–4.8 cm long; capsule with exocarp not separating from the adnate mesocarp and endocarp; endemic to Cuba . . . . . 3. *D. gnaphalantha*

10′. Corolla magenta or lavender, 4.5–8.8(rarely 10) cm long; capsule with exocarp separating from the adnate mesocarp and endocarp; Mexico and Nicaragua . . . . . 6. *D. laxiflora*

1. Distictis buccinatoria (DC.) A. H. Gentry, Brittonia 25: 237. 1973. *Pithecoctenium buccinatorium* DC. in A. DC., Prodr. 9: 195. 1845. *Pithecoctenium buccinatorium* var. *subinclusum* DC. in A. DC., Prodr. 9: 195. 1845. *Phaedranthus buccinatorius* (DC.) Miers, Proc. Roy. Hort. Soc. London 3: 182. 1863, "*buccinatorum*." TYPE: Mexico. s. loc., 1833, *M. Mairet de Chaudefont s.n.* (lectotype, designated here, G-DC not seen, microfiche G-DC 9.195.6!, photo F neg. 7655!).

Pithecoctenium buccinatorium var. *exsertum* DC. in A. DC., Prodr. 9: 195. 1845. *Phaedranthus exsertus* (DC.) Miers, Proc. Roy. Hort. Soc. London 3: 183. 1863. TYPE: Mexico. s. loc., 1832, *L. Alamán s.n.* (lectotype, designated here, G-DC not seen, microfiche G-DC 9.195.6!).

Bignonia ghiesbreghtii C. Heller ex Peyr., Linnaea 30: 45. 1859, "*giesbreghtii*." TYPE: Mexico. Toluca, 8200 ft., s.d., *C. Heller 390* (holotype, IB not seen; isotype, W!).

Phaedranthus lindleyanus Miers, Proc. Roy. Hort. Soc. London 3: 182. 1863. TYPE: Mexico. s. loc., "in hort. Soc. Hort. Cult., anno 1834," *W. Lambert s.n.* (holotype, CGE!).

Young branchlets weakly hexangular, the angles ribbed and sometimes detaching with age, golden, velutinous, villose, or glabrous with only nodes pilose, trichomes simple, glabrescent; pseudostipules foliaceous, ovate or lanceolate (rarely obovate), 5–35 × 5–28 mm, frequently persistent. Leaves bifoliolate, frequently with tendrils, tendrils usually trifid (rarely 5-fid), tips usually filamentous, infrequently disk-like; petiole 1–2.5 cm, velutinous, pilose or glabrous; petiolule 0.9–3 cm, velutinous, pilose or glabrous; leaflets lanceolate, 5–10.5 × 3–6.1 cm, membranous, 3 or 4 pairs of lateral veins, tertiary veins reticulate, midrib and lateral veins slightly impressed adaxially and prominent abaxially, adaxial surface drying greenish brown, densely soft puberulent overall or trichomes restricted to major veins, or glabrous overall, sometimes scattered lepidote, abaxial surface drying golden green, densely to scattered pilose or trichomes just along main veins and in nerve axils, densely pellucid-lepidote, usually with large glands scattered near base, base obliquely truncate (rarely obtuse), apex acute with tip apiculate. Inflorescence a raceme or racemose panicle, peduncle with rachis 4–24 cm with 4 to 17 flowers, peduncle, rachis, and pedicels villose or pilose, or peduncle and/or rachis glabrous, bracteoles affixed along length of pedicel, linear, 4–9 × 0.5–1.5 mm, early caducous; calyx 10–14 × 10–13 mm, coriaceous, densely golden tomen-

tose or pilose, external glandular fields short and broad, obscured by trichomes, apex denticulate; corolla opening with tube yellow and lobes red or orange-red, all turning red with age, weakly zygomorphic, gradually tubular-infundibular from cylindric base, (rarely 5.2)7.5–12 cm, subcoriaceous to coriaceous, externally golden tomentose above glabrous base, tube (rarely 4.4)7–9 cm with cylindric base (rarely 12)15–27 mm and mouth 14–28 mm wide, internally villose at insertion of stamens, otherwise glabrous, lobes 7–30 × 10–25 mm, externally without glandular fields at base, internally lepidote, papillose or minutely tomentose; stamens and staminode inserted 25–35 mm from base of corolla tube, shorter stamens with filaments 35–55 mm, included to exserted, longer stamens with filaments 50–65 mm, slightly exserted at mouth of corolla tube to 20 mm beyond, all anthers 5–7 mm, staminode 12–28 mm; disc 1.5–3 × 4–6 mm; pistil 7.3–8 cm, ovary ellipsoid or oblong, 7–8 × 4.5–5 mm, densely sericeous, style subexserted, pilose at base to lower half, stigma lanceolate to elliptic. Capsule oblong-elliptic, 13–15 × 4–5.5 cm, ca. 2.5 cm in diam., base acute, apex acute with tip rounded and retuse, valves convex but slightly compressed, exocarp not separating from the adnate mesocarp and endocarp at maturity, glabrous, surface with ± longitudinal wrinkles, midrib slightly raised apically, wall ca. 4 mm thick. Seeds 1–1.3 × 4–4.5 cm, body glabrous, the wings well demarcated from the seed body, off-white, membranous, hyaline.

Figure: Lindley (1830: plate 1301, as *Bignonia "cherere"*), Menninger et al. (1970: plate 39, as *Phaedranthus buccinatorius*).

Distribution. Native to central and southern Mexico, from San Luis Potosí and Jalisco to Chiapas. Found in rocky areas between 1700 and 2300 m elevation (Fig. 1A).

Phenology. Flowering April, May, June, and August. Fruiting collection examined was from cultivated material.

Common name. Mexican Blood Trumpet (Menninger et al., 1970).

Uses. Cultivated in tropical and subtropical areas of the world for its beautiful flowers. Menninger et al. (1970: 112) declared, "The blood trumpet is one of the loveliest and showiest of evergreen climbers in the warm gardens of the earth."

De Candolle (1845) published *Pithecoctenium buccinatorium* with two varieties, α, *P. buccinatorium* var. *subinclusum*, and β, *P. buccinatorium* var. *exsertum*. There has been some confusion as to the types of these names. For the species name de Candolle stated "in Mexico legit cl. *Mairet* Necomensis *Bignonia buccinatoria* Mair. in litt. 1833," and indicated that he had a herbarium specimen (de Candolle, 1845: 195). For *P. buccinatorium* var. *subinclusum*, he also indicated that he had a herbarium specimen but did not identify the collector, implying that the type of both variety *subinclusum* and the species was the same, *Mairet s.n.* (G-DC; de Candolle, 1845). For *P. buccinatorium* var. *exsertum*, no dried material was referred to, but he stated (de Candolle, 1845: 195) "cum prior a cl. *Mairet* comm.," suggesting that this was the "*Bignonia buccinatoria*" Mairet had collected. In Gentry's synopsis of *Distictis*, he cited the holotype of both *P. buccinatorium* and *P. buccinatorium* var. *exsertum* as "Mexico. *Mairet s.n.* (G-DC)" (Gentry, 1974a: 495) and did not include the name *P. buccinatorium* var. *subinclusum*. Microfiches from G-DC indicate that there are three elements in that herbarium labeled *Bignonia buccinatoria/Pithecoctenium buccinatorium*, none with variety indicated. One sheet is of a disarticulated flower bud, but no collector is indicated and it is dated 1838; the second sheet is of a flowering branchlet with two open flowers, the anthers not visible, and it is labeled "Mexique, *Mairet de la [Chaudefont].* 1833"; and the third sheet includes open flowers with the stamens far exserted and it is labeled "Mexique. *M. Alaman.* 1832." The *Alamán* collection matched de Candolle's (1845) description of *P. buccinatorium* var. *exsertum* and was, therefore, chosen as the lectotype of this variety and the *Mairet* collection chosen as the lectotype of the species and variety *subinclusum*. *P. buccinatorium* var. *exsertum* is treated here as a synonym of *P. buccinatorium*.

Type materials of *Bignonia ghiesbreghtii* and *Phaedranthus lindleyanus* were examined in this study, and, based on these observations, the species were placed in synonymy of *Distictis buccinatoria*, thereby following Gentry (1974a).

The preceding description is based largely on herbarium specimens taken from cultivated plants. The large, rather narrow, tubular-infundibular, orange-red corolla appears to be adapted toward hummingbird pollination. *Distictis buccinatoria* has been hybridized with *D. laxiflora* to form the cultivar *Distictis* cv. 'Rivers,' and marketed as "Distictis riversii" (a name with no scientific standing) (Menninger et al., 1970: plate 42). *Distictis* cv. 'Rivers' has large (9–9.5 cm long) corollas with purple lobes and a yellow tube and throat, and included stamens. Another form of the hybrid is also grown, with purple corollas ca. 5.5 cm long and exserted anthers. It is not known if *D. buccinatoria* and *D. laxiflora* hybridize naturally.

Distictis buccinatoria and *D. laxiflora* are the only species of *Distictis* found in Mexico. They can appear quite similar vegetatively and in flower, but the corollas of *D. laxiflora* are magenta or lavender, usually smaller than those of *D. buccinatoria* (4.5–8.8 cm long), and the stamens are included. The fruits of *D. laxiflora* are also smaller (8.5–10.5 cm long), more elliptic, and with the apex acute to acuminate, the tip not rounded and retuse, and the exocarp separates from the adnate mesocarp and endocarp at maturity. Vegetatively, *D. laxiflora* tends to have less persistent pseudostipules and smaller leaflets (2–8.5 × 1–6 cm) than *D. buccinatoria*.

Selected specimens examined. MEXICO. **Chiapas:** Pajal Ton, 2 km al N de la cabecera de Amatenango, *J. López P. 310* (MO). **Jalisco:** El Terrero, Mpio. Tonila, limestone plateau betw. Volcán de Colima and Sierra Madre de Manantlan, 32 km NW of city of Colima, *L. M. González V. et al. 3667* (photocopy and line drawing of IBUG at MO). **México:** Purificación, Texcoco, *E. Matuda 18859* (MO). **Morelos:** Mtns. above Tepoztlán, *O. Nagel 8001* (AMES). **Puebla:** s. loc., *D. Bilimek 230* (GH). **San Luis Potosí:** s. loc., *J. G. Schaffner 746* (GH). **Tlaxcala:** St. Ana, *C. Conzatti 929* (GH).

2. Distictis frutescens (DC.) A. Pool, comb. nov.
Pithecoctenium frutescens DC. in A. DC., Prodr. 9: 196. 1845. TYPE: Brazil. s. loc., 1832, *J. Lhotsky s.n.* (holotype, G-DC not seen, microfiche G-DC 196.15!, photo F neg. 7651!).

Pithecoctenium stipulare Mart. ex DC. in A. DC., Prodr. 9: 194. 1845. Syn. nov. *Anomoctenium stipulare* (Mart. ex DC.) Pichon, Bull. Soc. Bot. France 92: 227. 1946. *Distictis stipularis* (Mart. ex DC.) A. H. Gentry, Ann. Missouri Bot. Gard. 61: 498. 1974, non Mart. ex Glaz., 1911. TYPE: Brazil. Bahia, s.d., *C. F. Martius 2079* (lectotype, designated by Gentry, 1974a: 498, M!, photo K neg. 5949!).

Distictis stipularis Mart. ex Glaz., Bull. Soc. Bot. France 58 (Mém. 3f): 525. 1911. Syn. nov. TYPE: Brazil. Belem, dans les bourbiers, Rio-Jan., 1886, *A. Glaziou 8812* (holotype, P not seen; isotype, G!, K not seen).

Young branchlets sharply hexangular, the angles ribbed and detaching with age, with scattered to dense, ferruginous, dendritic trichomes less than 0.3 mm, some furfuraceous and without rachis mixed with ones with rachis, the trichome arms numerous, arms and internodes equally small; pseudostipules foliaceous, broadly ovate, 6–20 × 7–20 mm, persistent. Leaves bifoliolate frequently with tendrils, tendrils trifid, tips filamentous; petiole 1–2.6(rarely 3) cm, with trichomes like those of branchlet, especially dense adaxially; petiolule 0.3–1.2 cm, with trichomes like those of branchlet, especially dense adaxially; leaflets oblong-elliptic, 3–12 × 2–7.2 cm, subcoriaceous, 3 or 4 pairs of lateral veins, tertiary veins reticulate, midrib and lateral veins slightly impressed with tertiary veins flat or slightly prominent (rarely all flat) adaxially, midrib and lateral veins prominent abaxially with tertiary veins flat or slightly prominent, adaxial surface drying dark greenish brown or reddish brown, with trichomes on midrib like those of branchlet, sometimes scattered lepidote, abaxial surface drying dark to light brown-green, with dendritic trichomes along midrib and in basal lateral vein axils, trichomes white to tawny, less than 0.3 mm, but with fewer and longer arms than the trichomes found on branchlet (sometimes mixed with the branchlet-type trichomes), scattered pellucid-lepidote, occasionally with large glands, base obliquely rounded to subcordate, apex acute to rounded with tip apiculate and usually curving. Inflorescence a raceme, peduncle with rachis 4–12.5 cm with 2 to 8 flowers, peduncle, rachis, and pedicels with dense trichomes like those of branchlet, bracteoles affixed at base of pedicel, oblong or elliptic, 13–22 × 2–8 mm, persistent; calyx 5–8 × 6–8 mm, subcoriaceous, tomentose with furfuraceous-dendritic trichomes strongly appressed, rarely with a few trichomes with rachis, external glandular fields short and broad, apex denticulate; corolla with tube externally cream to yellow and internally yellow to orange and lobes lilac to mauve, weakly zygomorphic, gradually tubular-infundibular or tubular-campanulate from cylindric base, 5.5–10.2 cm, subcoriaceous to membranous, externally with scattered to dense minute dendritic trichomes above glabrous base, tube 4.5–7.5 cm with cylindric base 10–15 mm and mouth 15–30 mm wide, internally villose at insertion of stamens, otherwise glabrous, lobes 15–25 × 15–35 mm, externally with linear glandular fields at base, internally lepidote; stamens and staminode inserted 12–20 mm from base of corolla tube, shorter stamens with filaments 12–20 mm, included, longer stamens with filaments (rarely 20)27–30 mm, included, all anthers 3–4.5 mm, staminode 4–8 mm; disc ca. (1.5)2 × 4 mm; pistil 5–5.6 cm, ovary ovoid, ca. (3)4 × 2 mm, densely short velutinous with simple trichomes at base mixed with small dendritic trichomes apically, style included, with dendritic trichomes at base to lower 2/3, stigma oblong. Capsule wide elliptic, 4–8 × 2–4 cm, base attenuate, apex acute with apiculate tip, puberulent-tomentose. Seeds small winged, dark brown.

Figure: Gomes (1952: plate 4, as *Distictella nigrescens* (Bureau & K. Schum.) J. C. Gomes).

Distribution. Eastern coast of Brazil, from Paraíba to Rio de Janeiro. Found in coastal vegetation or edge of wet forest, often along rivers, near sea level (Fig. 1B).

Phenology. Flowering August, October, November, and March. Fruiting collections not seen.

No reference to common name or economic use was found for this species.

Type materials of *Pithecoctenium frutescens* DC., *P. stipulare* Mart. ex DC., and *Distictis stipularis* Mart. ex Glaz. were examined in this study and found to be synonymous. In publishing *D. stipularis*, Glaziou (1911: 525) cited "*D. stipularis* Mart. sp. n. ? in herb. Mus. Paris.–Belem, dans les bourbiers, Rio-Jan., no *8812*. Haute liane, fl. violet foncé." It might be argued that Glaziou intended to transfer *P. stipulare* Mart. ex DC. to *Distictis* based on material that he had seen at P annotated "*Distictis stipularis* Mart." However, by citing "sp. n.?" it seems clear that he did not know that this species had been published. It might be suggested that as both de Candolle (1845) and Glaziou (1911) employed the same epithet and Glaziou (1911) referred to material at P, Article 33.3 of the *International Code of Botanical Nomenclature* (McNeill et al., 2006: 63) as illustrated in Ex. 8 applies. However, for a species with such large pseudostipules, the use of the same epithet "stipularis" could easily be coincidental. In addition, we do not know the material Glaziou saw at P (Glaziou, 1911) to be duplicates of the material that de Candolle saw at M (de Candolle, 1845), as there are a number of other candidates, as cited in Bureau and Schumann (1896), and as Glaziou (1911) specifically cited his own number, *8812*. It seems, therefore, that application of Article 33.3 of the *International Code of Botanical Nomenclature* (McNeill et al., 2006) is too great a stretch. It might also be argued that Glaziou's (1911) description is not diagnostic (Article 32.1, Ex. 3 in McNeill et al., 2006). However, the other species included are described as sarmentose shrubs with white or yellow flowers. Therefore, it is proposed that *D. stipularis* Mart. ex Glaz. was validly published as a new taxon and that Gentry's (1974a) combination *D. stipularis* (Mart. ex DC.) A. H. Gentry is a later homonym of *D. stipularis* Mart. ex Glaz. (Glaziou, 1911) and therefore illegitimate (Article 53.1 in McNeill et al., 2006), thus necessitating the new combination, *D. frutescens* (DC.) A. Pool. This is not a decision made lightly, as the names *P. stipulare* Mart. ex DC., *Anomoctenium stipulare* (Mart. ex DC.) Pichon, and *D. stipularis* (Mart. ex DC.) A. H. Gentry have been steadily employed since the time of de Candolle for this species of somewhat limited distribution and no known use, while *P. frutescens* DC. has been ignored.

The syntypes of *Anemopaegma nigrescens* Bureau & K. Schum. (and *Distictella nigrescens* (Bureau & K. Schum.) J. C. Gomes), *F. Sello 398* (B) and "idem *L.*" [= *Lhotzky*?] *313* (B), were not seen in this study and are presumed destroyed. However, duplicates, while not definitely seen, probably exist. Gentry (1974a) proposed that the "idem *L.*" of Bureau and Schumann (1896: 126) referred to *Luschnath*, but it seems more probable that it stands for *Lhotzky* (a collection of Lhotzky was cited below the preceding species), and it is even possible that this *L. 313* at B is a duplicate of the holotype of *Pithecoctenium frutescens*, *Lhotsky s.n.* (G-DC). In this case, *A. nigrescens* would be a superfluous name for *P. frutescens*, a name not included in Bureau and Schumann (1896). It seems premature to lectotypify this name; however, the placement of the taxon seems clear. Not only is the original description of Bureau and Schumann (1896) for *A. nigrescens* Bureau & K. Schum. very similar to their description of *P. stipulare* Mart. ex DC., but they also cite one of the syntypes of *A. nigrescens*, *Sello 398*, under *P. stipulare* (Bureau & Schumann, 1896). This last argument was previously presented by Sandwith (1965) who placed *A. nigrescens* Bureau & K. Schum. and *Distictella nigrescens* (Bureau & K. Schum.) J. C. Gomes in synonymy of *P. stipulare*. Gentry treated *A. nigrescens* and *Distictella nigrescens* as synonyms of *Distictis stipularis*. They are considered here to be synonyms of *Distictis frutescens*; however, as the syntypes of *A. nigrescens* have not been examined in this study, the names are not listed in formal synonymy.

Fruiting material and seeds were not seen in this study; descriptions here are adapted from Gomes (1952). Gomes' (1952) description of the seed as small winged and dark brown suggests that it might be similar to the seed of *Distictis occidentalis*, which has a corky seed body and small coriaceous wings poorly demarcated from the seed body, modifications associated with water dispersal.

Distictis frutescens, *D. granulosa*, *D. occidentalis*, *D. scabriuscula*, and *D. steyermarkii* possess dendroid trichomes on many parts and rows of glands on the external surface of the corolla at the base of the corolla lobes, features not found in the other species of *Distictis*. *Distictis frutescens* differs from these other species in having large foliaceous pseudostipules (6–20 × 7–20 mm), while the others either lack pseudostipules or have very small, inconspicuous ones (2–8 × 1.5–5 mm) and larger, foliaceous bracteoles (13–22 × 2–8 mm vs. 1.5–12 × 0.5–2 mm).

Selected specimens examined. BRAZIL. **Bahia:** Mpio. Caravelas, Juerana, *G. Hatschbach & F. J. Zelma 50752* (MO); coastal zone, 11 km S of Santa Cruz, Cabrália, *R. M. Harley et al. 17088* (photograph of K at MO). **Espírito Santo:** Mpio. São Mateus, Rio Preto do Sul, BR-101, *G. Hatschbach & O. Guimaraes 46986* (MO); Goitacazes, Rio Doce, *J. G. Kuhlmann 6532* (MO). **Minas Gerais:** s. loc., *Ackermann s.n.* (BR). **Paraíba:** Mpio. Rio Tinto, BR-101, proximo ao Rio Muriri, *L. G. Lohmann et al. 695* (MO). **Rio de Janeiro:** Campos, Rio Paraiba, *E. P. Heringer 1306* (MO).

3. Distictis gnaphalantha (A. Rich.) Greenm., Proc. Amer. Acad. Arts 33: 487. 1898. *Bignonia gnaphalantha* A. Rich. in Sagra, Hist. Fis. Cuba, Bot. 11: 105. 1850. *Distictis gnaphalantha* (A. Rich.) Urb., Repert. Spec. Nov. Regni Veg. 14: 310. 1916, nom. illeg. TYPE: Cuba. La Habana, s.d., *R. de la Sagra s.n.* (holotype, P not seen, digitized image!; isotypes, P not seen, digitized image! [2]).

Distictis rhynchocarpa Urb., Symb. Antill. 9: 253. 1924. *Distictis gnaphalantha* subsp. *rhynchocarpa* (Urb.) Borhidi & O. Muñiz, Bot. Közlem. 58: 176. 1971. TYPE: Cuba. Oriente: Santiago de Cuba, *E. L. Ekman 1388* (holotype, S not seen).

Young branchlets generally sharply hexangular, the angles ribbed and detaching with age, densely puberulent with simple trichomes; pseudostipules foliaceous, suborbicular, 3–23 × 3–20 mm, usually persistent. Leaves bifoliolate, frequently with tendrils, tendrils trifid, tips often disk-like; petiole 0.4–2.4 cm, densely puberulent to pilose; petiolule 0.1–0.7 cm, densely puberulent to pilose; leaflets broadly elliptic to elliptic or oblong, 1.7–6.3 × 1.2–4.4 cm, membranous, 3 or 4 pairs of lateral veins, tertiary veins reticulate, midrib, lateral and tertiary veins impressed adaxially (or tertiary veins flat), midrib and lateral veins prominent abaxially, adaxial surface drying dark olive-green to almost black, puberulent overall or at least on main veins, conspicuously pellucid-lepidote, abaxial surface drying yellow-green to dark green, puberulent overall or at least on main veins at base, densely pellucid-lepidote, sometimes with large glands scattered but especially dense in lower lateral vein axils, base obliquely truncate, apex rounded to acute with tip apiculate or retuse. Inflorescence a panicle, peduncle with rachis 1–17 cm with 2 to 10 flowers, peduncle, rachis, and pedicels densely puberulent and lepidote, bracteoles affixed at base of pedicel, linear, ca. 4 × 0.5 mm, early caducous; calyx 4–7 × 5–6 mm, membranous to subcoriaceous, pubescent with minute appressed trichomes, lepidote, external glands scattered or arranged in short, broad glandular fields, apex generally denticulate; corolla white, weakly zygomorphic, gradually tubular-infundibular to tubular-campanulate from cylindric base, 3.5–4.8 cm, membranous to subcoriaceous, externally lepidote or tomentose and lepidote above glabrous base, tube 2.8–3.5 cm with cylindric base 5–9 mm and mouth 10–16 mm wide, internally with ring of trichomes at insertion of stamens, otherwise glabrous, lobes 7–12 × 8–13 mm, externally without glandular fields at base, internally with trichomes scattered to dense; stamens and staminode inserted ca. 11 mm from base of corolla tube, shorter stamens with filaments ca. 13 mm, included, longer stamens with filaments 17–20 mm, included, all anthers ca. 3 mm, staminode ca. 4 mm; disc ca. 2 × 3 mm; pistil 3.1–3.2 cm, ovary ovoid to oblong, 3–4 × 2–2.5 mm, densely sericeous, style included, pubescent along basal half, stigma oblong. Capsule elliptic, 3–7 × 1.5–2.5 cm, base acute and truncate, apex acuminate, valves convex, exocarp not separating from the adnate mesocarp and endocarp at maturity, glabrous (pubescent when young), surface warty-glandular, midrib subevident to impressed, wall ca. 1 mm thick. Seeds not seen.

Figure: Liogier (1957: fig. 182).

Distribution. Endemic to Cuba. Found in forest over limestone between 100 and 300 m elevation (Fig. 1A).

Phenology. Flowering February to July.

Common names. Bejuco logarti (Liogier, 1957; under *Distictis rhynchocarpa*).

No reference to economic use was found for this species.

Urban (1916a) has been credited with transferring *Bignonia gnaphalantha* to *Distictis* (Liogier, 1957; Gentry, 1974a); however, the transfer had been made previously by Greenman (1898).

Urban (1924) published the new species *Distictis rhynchocarpa* Urb., and Borhidi and Muñiz (1971) treated *D. rhynchocarpa* as a subspecies of *D. gnaphalantha*, distinguishing it from *D. gnaphalantha* based on its retuse versus apiculate leaflet apex. As this character is very variable and can even vary on leaflets of the same specimen, *D. rhynchocarpa* is considered here to be a synonym of *D. gnaphalantha*. This follows Gentry (1974a).

Only two species of *Distictis* are found on Cuba, *D. lactiflora* and *D. gnaphalantha*. They have very similar flowers, differing primarily in the lepidote (and usually otherwise glabrous) ovary of *D. lactiflora* versus the densely sericeous one of *D. gnaphalantha*. Vegetatively, *D. lactiflora* differs in having small, narrow, caducous pseudostipules (3–5 × 1–2.2 mm) and leaflets that are coriaceous with venation conspicuously prominent on the adaxial surface and glabrous on the abaxial surface. *Distictis gnaphalantha* is similar to *D. laxiflora* vegetatively, but *D. laxiflora* (from Mexico and Nicaragua) has larger (4.5–8.8 cm), magenta or lavender corollas.

No complete mature fruit of *Distictis gnaphalantha* were seen in this study. The fruit description is based on one old valve and one immature fruit of *C. Wright 3050*, and from Liogier (1957), and Urban (1924).

Selected specimens examined. CUBA. s. loc., *C. Wright 3050* (MO). **La Habana:** Escaleras de Jaruco, ca. 60 km SE

of Habana, *A. H. Gentry 50829* (MO). **Oriente:** Antilla (ad Nipe Bay), *E. L. Ekman 7324* (MO); Santiago de Cuba, *E. L. Ekman 19185* (MO). **Santa Clara:** Cienfuegos, *R. Combs 106* (MO).

4. Distictis granulosa Bureau & K. Schum. in Mart., Fl. Bras. 8(2): 179. 1896. *Distictella granulosa* (Bureau & K. Schum.) Urb., Repert. Spec. Nov. Regni Veg. 14: 310. 1916. *Pithecoctenium granulosum* (Bureau & K. Schum.) Sprague & Sandwith, Bull. Misc. Inform. Kew 1932: 89. 1932. *Anomoctenium granulosum* (Bureau & K. Schum.) Sandwith, Kew Bull. 19: 413. 1965. TYPE: Guyana. s. loc., s.d., *R. H. Schomburgk 229 S* (neotype, designated here, K not seen, digitized image!, photo K neg. 4302 not seen).

Pithecoctenium uleanum Kraenzl., Notizbl. Königl. Bot. Gart. Berlin 6: 377. 1915. TYPE: Brazil. Rio Branco (Terr. Roraima), Nov. 1911, *E. Ule 7706* (lectotype, designated here, G not seen, photo F neg. 26202!).

Young branchlets sharply hexangular, the angles ribbed and detaching with age, with scattered to dense, golden to ferruginous, dendritic trichomes to 0.15 mm, some furfuraceous and without rachis, sometimes mixed with ones with rachis, the trichome arms usually numerous (rarely less than 4), occasionally longer than the internodes; pseudostipules absent or rarely present, not foliaceous, oblong, 2–4 × 1.5–2 mm, caducous. Leaves bifoliolate, often with tendrils, tendrils trifid, tips filamentous; petiole 0.6–4 cm, with trichomes like those of branchlet; petiolule 0.5–2.5 cm, with trichomes like those of branchlet; leaflets obovate or oblong-elliptic (rarely broad elliptic), 7–19 × 3.5–10.5 cm, subcoriaceous to coriaceous, 3 or 4 pairs of lateral veins, tertiary veins mostly parallel and ladder-like, rarely weakly reticulate, midrib and lateral veins impressed adaxially and tertiary veins flat (or rarely tertiary veins impressed), midrib and lateral veins prominent abaxially with tertiary veins flat or prominent, adaxial surface usually drying reddish brown (rarely brownish green), with trichomes on midrib like those of branchlet (rarely glabrous), sometimes scattered lepidote, abaxial surface drying olive-green often with grayish cast, with trichomes like those of branchlet occasionally scattered on surface, usually restricted to midrib or midrib and lateral veins, scattered pellucid-lepidote, occasionally with large glands, base obliquely obtuse or rounded, or cuneate and then rounded, apex obtuse with tip cuspidate and apiculate, and often curving, rarely truncate and tip cuspidate or retuse. Inflorescence a raceme, peduncle with rachis 6–18 cm with 8 to 16 flowers, usually fragile and breaking in collections, peduncle, rachis, and pedicels with trichomes like those of branchlet, bracteoles affixed at base of pedicel, broadly oblong or spatulate, 1.5–2 × 1 mm, usually persistent; calyx (rarely 5)6–10 × (rarely 6)8–11 mm, subcoriaceous, tomentose with furfuraceous-dendritic trichomes strongly appressed, external glandular fields narrow and oblong, apex denticulate (rarely truncate); corolla with tube externally yellow or white and internally yellow and lobes purple or lavender, weakly zygomorphic, gradually tubular-infundibular or tubular-campanulate from cylindric base, (rarely 5.5)6.2–9 cm, subcoriaceous to membranous, externally with dense minute dendritic trichomes above glabrous base, tube (rarely 4)4.5–6.5(rarely 7) cm with cylindric base 8–15 mm and mouth 20–25 mm wide, internally villose at insertion of stamens, otherwise glabrous, lobes (rarely 10)15–30 × 20–35 mm, externally with linear glandular fields at base, internally with scattered minute dendritic trichomes; stamens and staminode inserted 11–12(rarely 14) mm from base of corolla tube, shorter stamens with filaments 15–21 mm, included, longer stamens with filaments 19–31 mm, included, all anthers (rarely 3)3.5–5 mm, staminode 2–5 mm; disc 2–2.2 × 4.2–5 mm; pistil 4.7–5 cm, ovary ovoid, (rarely 3)4–4.5 × 2–2.5 mm, densely tomentose with furfuraceous-dendritic trichomes, style included, with dendritic trichomes at base to lower 3/4, stigma narrow elliptic. Capsule elliptic, (rarely 7)8.5–11 × (rarely 2.5)3–4.5 cm, ca. 0.5 cm in diam., base acute and truncate, apex acuminate or acute, valves convex, exocarp not separating from the adnate mesocarp and endocarp at maturity, densely furfuraceous-dendritic appressed tomentose, scattered large glands, midrib impressed or slightly prominent, wall ca. 1 mm thick. Seeds (rarely 1)1.2–2.7 × (rarely 2.8)3.3–4.8 cm, body pubescent, the wings well demarcated from the seed body, tawny, membranous, hyaline.

Distribution. Northern South America: Colombia (Gentry, 1982b), along the border of Venezuela with Colombia, Apure, Bolívar, and Amazonas in Venezuela, throughout the Guianas (from Guyana, known only from type), northern Brazil bordering the Amazon and its tributaries, Madre de Dios and Puno in Peru, and La Paz, Bolivia. Found in moist or wet forest, sometimes disturbed, frequently characterized as terra firme on sandy soil, between 30 and 1200 m elevation (Fig. 2B).

Phenology. Flowering: January, February, March, May, July, September, October, November, and December, and fruiting March, July, September, November, and December.

No reference to common name or economic use was found for this species.

Bureau and Schumann (1896) published *Distictis granulosa* citing only *M. R. Schomburgk 400*, which was housed at B and is presumed destroyed. Gentry (1982b) reported an isotype of *M. R. Schomburgk 400* at K; however, the staff at K was unable to find this specimen in 2006 and believe that they do not have it. Sprague and Sandwith (1932b) published the combination *Pithecoctenium granulosum* (Bureau & K. Schum.) Sprague & Sandwith, citing *M. R. Schomburgk 400* (B), *R. H. Schomburgk 229 S* (K), and *Ule 7706* (K). Judging from their treatment of other taxa in this paper, it appears that they have cited all material for the taxa treated, including herbaria, and that they studied material from B, BM, BR, GOET, K, and P. If they had seen *M. R. Schomburgk 400* from herbaria other than B, they would have most likely included this information. This, in addition to the absence of actual specimens of *M. R. Schomburgk 400* in the database of the Schomburgks' collections compiled by the Nationaal Herbarium Nederland (<http://www.nationaalherbarium.nl/>), suggests that there are no extant specimens of *M. R. Schomburgk 400*, necessitating the selection of a neotype. *R. H. Schomburgk 229 S* (K) is the most logical choice for a neotype: in 1931, Sprague and Sandwith identified *R. H. Schomburgk 229 S* (K) as *Pithecoctenium granulosum* (Bureau & K. Schum.) Sprague & Sandwith (= *Distictis granulosa* Bureau & K. Schum.) and annotated the sheet as "compared with the type at B." It is also known that M. R. Schomburgk and R. H. Schomburgk collected together in Guyana (Stafleu & Cowan, 1985; <http://www.nationaalherbarium.nl/>), increasing the likelihood of *M. R. Schomburgk 400* (B) and *R. H. Schomburgk 229 S* (K) having been collected at the same locality and even, perhaps, being virtual duplicates. The latter has been implied in other instances (e.g., Sprague & Sandwith, 1932a: 21; in which *M. R. Schomburgk 980* = *R. H. Schomburgk 641* and *M. R. Schomburgk 928* = *R. H. Schomburgk 658*).

The holotype of *Pithecoctenium uleanum* Kraenzl. at B is presumed destroyed. A photo of an isotype of *P. uleanum* at G was observed, the specimen at G chosen as the lectotye, and the name placed in synonymy of *Distictis granulosa*, thereby following the synonymy of Gentry (1974a).

Kränzlin (1921:118) published the name *Distictis rosea* Kraenzl. and Sampaio (1935: 118) transferred *D. rosea* to *Distictella*. The holotype, *Ule 53b* (Brazil. Amazonas: Marary Jusua, 5 Sep. 1900), was at B and is presumed destroyed. No duplicates were seen in this study, but the digitized image of a photo of the holotype (K neg 696) was seen and *D. rosea*, based on this image and the original description, can be assigned to the synonymy of *Distictis granulosa*. A neotype has not been chosen as Ule specimens are widely distributed and it is hoped that a duplicate of *Ule 53b* will eventually be found.

Distictis granulosa is very similar to both *D. frutescens* and *D. scabriuscula*, all of which have purple corolla lobes, dendritic trichomes, and glandular fields at the base of the corolla lobes. *Distictis frutescens* differs from the other two in having large foliaceous pseudostipules (6–20 × 7–20 mm), the tertiary veins of the leaflets reticulate versus generally ladder-like, the calyx denticules more distinct, and the calyx glandular fields somewhat broader. *Distictis scabriuscula* and *D. granulosa* are very similar but can be separated by several, sometimes overlapping characteristics. The trichomes of *D. scabriuscula* are longer than those of *D. granulosa* (to 0.3 mm vs. 0.15 mm long) and generally more dense and persistent, but as these trichomes tend to mat, and in both cases the trichomes are very small, this is not the most useful character to use for purposes of identification. The leaflets of *D. scabriuscula* are membranous to chartaceous, elliptic, and often smaller (4–10.2 × 2–6.2 cm) than the coriaceous, often obovate, and larger (7–19 × 3.5–10.5 cm) leaflets of *D. granulosa*. In addition, the midrib and lateral veins of the leaflets of *D. scabriuscula* are flat on the adaxial surface versus impressed in *D. granulosa*. The bracteoles of *D. scabriuscula* are caducous, narrow, and long (6–11 × 0.5–1 mm) versus much more persistent, short, and relatively broad (1.5–2 × 1 mm) in *D. granulosa*. Finally, *D. scabriuscula* appears to be restricted to central-eastern Brazil, where *D. granulosa* has not been found.

Prior to Gentry (1992), specimens of *Distictis* with white flowers from northwestern South America were included in *D. granulosa*. Gentry (1992) recognized these white-flowered *Distictis* as a new species, *D. occidentalis*. The two are similar in type and distribution of dendritic trichomes, the shape, size, and persistence of pseudostipules, the leaflet size, shape, and ladder-like pattern of tertiary veins, the bracteole duration, overall flower size, calyx apex, and presence of corolla glandular fields. *Distictis occidentalis* differs from *D. granulosa* in the color of the corolla lobes (white vs. purple or lavender), leaflets chartaceous to membranous (vs. subcoriaceous to coriaceous) with lateral veins flat on the upper surface (vs. impressed), short and broad calyx glandular fields (vs. oblong), and seed wings that are very reduced and coriaceous-opaque (vs. well developed, membranous and hyaline). According to Gentry (1992), the two species overlap at Tambopata, Peru, with *D. occidentalis* occurring in swampy areas around lake margins and *D. granulosa* occurring in sandy upland forests.

Selected specimens examined. BOLIVIA. **La Paz:** Abel Iturralde, Province Alto Madidi, across from mouth of Río Enlatagua, *A. Gentry & S. Estensoro 70258* (MO). BRAZIL. **Amazonas:** Mpio. de Tefé, estrada do aeroporto, *I. L. Amaral et al. 160* (MO); Manaus–Caracarai Hwy., Km 50, BR 174, *G. T. Prance et al. 22656A* (MO). **Pará:** Macau airstrip, 1 1/2 hours upstream from Lageira airstrip on Rio Maicuru, *J. J. Strudwick et al. 3464* (MO); Senador José Porfirio (Sozel), margem direita do Rio Xingu, *G. dos Santos et al. 279* (MO). **Roraima:** BR 174 Manaus–Venezuela Hwy., Malacacheta, E of Boa Vista, *W. C. Steward et al. 253* (MO). FRENCH GUIANA. St. Laurent piste de paul Isnard, *C. Feuillet 1458* (MO). PERU. **Madre de Dios:** Tambopata, Explorer's Inn Tourist Camp at jct. of Ríos La Torre and Tambopata, 4, *H. Gentry et al. 51393* (MO). **Puno:** Ridge betw. Río Candamo and Río Guacamayo, *A. Gentry et al. 76932* (MO). SURINAME. **Sipaliwini:** Voltzberg Nature Reserve, vic. of Voltzberg base camp, *B. Hoffman et al. 5310* (MO). VENEZUELA. **Amazonas:** Dept. Río Negro, Neblina Massif, Canyon Grande, along Río Mawarinuma betw. mouth of canyon and first major fork of river, ca. 7 airline km ENE of Puerto Chimo, *G. Davidse & J. S. Miller 27320* (MO). **Apure:** Dtto. Páez: Selva de Cutufí, betw. Cutufí on the Río Cutufí and the Río Sanare, *G. Davidse & A. C. González 21917* (MO). **Bolívar:** Tumeremo to Anacoco (N side of Cuyuni River), 64 km from Guyana frontier at Anacoco, *A. H. Gentry et al. 10657* (MO).

5. Distictis lactiflora (Vahl) DC. in A. DC., Prodr. 9: 191. 1845. *Bignonia lactiflora* Vahl, Symb. Bot. 3: 80, t. 66. 1794. *Macrodiscus lactiflorus* (Vahl) Bureau ex K. Schum. in Engl., Nat. Pflanzenfam. 4(3b): 216. 1894, "*lactiflora*." TYPE: St. Croix. s. loc., s.d., *F. A. F. C. West s.n.* (holotype, C not seen; isotype, BM!).

Bignonia rigescens Jacq., Pl. Hort. Schoenbr. 2: 44, t. 210. 1797. *Distictis rigescens* (Jacq.) DC. in A. DC., Prodr. 9: 191. 1845. *Macrodiscus rigescens* (Jacq.) Bureau. Monogr. Bignon. Atlas 13, t. 11. 1864. TYPE: tab. 210 in Jacq., Pl. Hort. Schoenbr. 2. 1797 (lectotype, designated here, tab. 210 in Jacq., 1797!).

Young branchlets sharply hexangular, the angles ribbed and detaching with age, densely puberulent or with few, small, simple trichomes, especially at nodes; pseudostipules not foliaceous, linear or elliptic, 3–5 × 1–2.2 mm, generally caducous. Leaves bifoliolate frequently with tendrils, tendrils trifid, tips often disk-like; petiole 0.5–2 cm, puberulent to pilose at least adaxially; petiolule 0.2–0.9 cm, puberulent to pilose at least adaxially; leaflets broadly elliptic or nearly orbicular to narrow-elliptic, 2–6 × 0.9–5 cm, subcoriaceous to coriaceous, 3 or 4(rarely 5) pairs of lateral veins, tertiary veins reticulate, midrib, lateral and tertiary veins conspicuously prominent on both surfaces but especially adaxially, adaxial surface drying yellow-green, glabrous, or puberulent on main veins, sometimes conspicuously pellucid-lepidote, abaxial surface drying yellow-green, glabrous, densely pellucid-lepidote, usually with large glands scattered but especially dense in lower lateral vein axils, base obliquely obtuse or rounded, apex rounded to acute or acuminate with tip retuse (rarely tip apiculate). Inflorescence a panicle (rarely raceme), peduncle with rachis 4–21 cm with 4 to 22 flowers, peduncle, rachis, and pedicels densely to scattered pubescent with small, appressed trichomes, bracteoles not known, early caducous; calyx 3–8 × 5–8 mm, membranous to subcoriaceous, pubescent with minute appressed trichomes to glabrous, lepidote, sometimes with scattered external glands or external glands arranged in short and broad glandular fields, apex truncate or rarely minutely denticulate; corolla pure white or white with pink or lavender flush, with yellow throat, weakly zygomorphic, gradually tubular-infundibular to tubular-campanulate from cylindric base, 3–5.5 cm, membranous to subcoriaceous, externally tomentose or puberulent with minute trichomes above glabrous base, tube 2.5–4 cm with cylindric base 5–10 mm and mouth 10–17 mm wide, internally with tufts of minute trichomes at insertion of stamens, otherwise glabrous, lobes 5–12 × 7–15 mm, externally without glandular fields at base, internally lepidote or minutely tomentose or papillose; stamens and staminode inserted 5–10 mm from base of corolla tube, shorter stamens with filaments 8–13 mm, included, longer stamens with filaments 14–18 mm, included, all anthers 2.7–3 mm, staminode 2–6 mm; disc 1–2 × 2.2–4 mm; pistil 2.1–3.2 cm, ovary ovoid or oblong, 3–4 × 1.5–2 mm, densely lepidote (rarely lepidote and puberulent), style included, glabrous, stigma elliptic to narrow elliptic. Capsule narrow elliptic to elliptic-fusiform, 4–9.9 × 1.5–2.7 cm, ca. 0.5 cm in diam., base acute and truncate, apex acuminate, valves convex, exocarp not separating from the adnate mesocarp and endocarp at maturity, glabrous, surface smooth, midrib subevident to impressed, wall ca. 1 mm thick. Seeds 0.6–1 × 1.3–2.3 cm, body glabrous or slightly papillose, the wings well demarcated from the seed body, light brown, membranous, hyaline.

Figure: Liogier (1995a: fig. 129-7).

Distribution. Cuba, Haiti, Dominican Republic, Puerto Rico, St. Croix, and St. Thomas (Liogier, 1957). Found in dry forests, dry roadside scrub, or dry coastal scrub, often on limestone, between sea level and 1000 m elevation (Fig. 2A).

Phenology. Flowering December to July and September; fruiting January, April, July, and October.

Common names. Liana fragante, Pegapalo, Viuda (Puerto Rico; Liogier, 1995a), Bejuco culebra, Liane blanche (Hispaniola; Liogier, 1995b).

Uses. Plants cultivated under this name are probably *Distictis laxiflora*; see discussion below the latter species.

Jacquin (1797) published *Bignonia rigescens* Jacq. with an informative description and excellent plate but without type citation, beyond the indication that it was from Caracas, and the latter citation is probably in error (Gentry, 1974a). No type specimen was found in this study or by Gentry (1974a), and apparently not by Britton (1925) or Bureau (1864), who cite the distribution as from the Antilles with no mention of a type from Caracas. In lieu of an actual specimen, the plate 210 is chosen here as the lectotype. The description and plate show that *B. rigescens* is synonymous with *Distictis lactiflora*, the only problems with this interpretation being that the stems are described as terete (vs. hexangular), but illustrated with definite angles, the leaf tendrils are illustrated as simple (vs. trifid), and it is cited as being from Caracas (*D. lactiflora* is known only from the West Indies). I follow Britton (1925), Gentry (1974a), and Liogier (1957, 1995a) in placing *B. rigescens* Jacq. in synonymy of *D. lactiflora* and assume that the Caracas citation is in error.

Bello (1881) published *Bignonia odorata* Bello, without indication of type (beyond country, Puerto Rico). The type is presumed to have been deposited at B and destroyed. *Bignonia odorata* was treated as synonymous with *Distictis lactiflora* by Bureau (1911), Britton (1925), Gentry (1974a), and Liogier (1995a). Judging from the description of Bello (1881), *B. odorata* is probably synonymous with *D. lactiflora*.

Vegetatively, *Distictis lactiflora* is similar to *D. staminea* in pseudostipule size, shape, and persistence, and leaflet size, texture, venation prominence, and apices. However, *D. staminea* differs from *D. lactiflora* in having leaflets that are pilose along the main veins abaxially and flowers that are orange and strongly bilabiate with the stamens exserted. The flowers of *D. lactiflora* are similar to those of *D. gnaphalantha*, but the latter has densely sericeous ovaries, foliaceous, persistent pseudostipules, and membranous leaflets with the veins impressed on the adaxial surface.

Selected specimens examined. CUBA. **Camaguëy:** Pastelillo, *E. L. Ekman 15549* (MO). DOMINICAN REPUBLIC. **Barahona:** 2 km S of Juancho, 26 km SW of Paraíso, *A. H. Gentry & M. Mejía 50736* (MO). **Peravia:** N of Baní, 13 km N of Cruce com Sanchez, *A. H. Gentry & M. Mejía 50814* (MO). HAITI. **Ouest:** Vic. of Cabaret, Baie des Moustiques, *E. C. Leonard & G. M. Leonard 12083* (MO). PUERTO RICO. **Guayama:** Salinas, *J. S. Miller & C. D. Sherman 6371* (MO). ST. CROIX. West-end, *J. J. Ricksecker 96* (MO).

6. Distictis laxiflora (DC.) Greenm., Proc. Amer. Acad. Arts 33: 486. 1898. *Pithecoctenium laxiflorum* DC. in A. DC., Prodr. 9: 195. 1845. TYPE: Mexico. Oaxaca, Julio, *G. Andrieux 220* (holotype, G-DC not seen, microfiche G-DC 9.195.7!; isotypes, M!, W!, photo F neg. 32869!).

Pithecoctenium cinereum DC. in A. DC., Prodr. 9: 195. 1845. *Distictis cinerea* (DC.) Greenm., Proc. Amer. Acad. Arts 33: 487. 1898. TYPE: Mexico, s. loc., 1833, *M. Mairet de Chaudefont s.n.* (lectotype, designated here, G-DC not seen, microfiche G-DC 9.195.10!, photo F neg. 7653!).

Pithecoctenium cinereum var. *parviflorum* Hochr., Bull. New York Bot. Gard. 6: 287. 1910. Syn. nov. TYPE: Mexico, Rocha, s. coll., s.n. (holotype, NY!).

Young branchlets weakly to strongly hexangular, the angles ribbed and sometimes detaching with age, scattered to densely white villose or pilose, with simple trichomes; pseudostipules foliaceous, orbicular or elliptic, 3–25 × 3–17 mm, usually caducous. Leaves bifoliolate, occasionally with tendrils, tendrils trifid, tips filamentous; petiole 0.7–2 cm, pilose or villose; petiolule 0.5–3 cm, villose or pilose; leaflets ovate or elliptic, 2–8.5 × 1–6 cm, membranous, 3 or 4 pairs of lateral veins, tertiary veins reticulate, midrib and lateral veins flat or slightly impressed adaxially and prominent abaxially, adaxial surface drying brownish green, puberulent to pilose or trichomes restricted to main veins, densely pellucid-lepidote, abaxial surface drying brownish green, pilose to villose, trichomes dense to scattered, or trichomes restricted to main veins and lateral vein axils, densely pellucid-lepidote, usually with large glands scattered but especially dense in lower lateral vein axils, base obliquely obtuse or rounded, apex acute, often with apiculate tip. Inflorescence a panicle, peduncle with rachis 6–12.5 cm with 6 to 16 flowers, peduncle, rachis, and pedicels villose or pilose (rarely trichomes only at nodes), bracteoles unknown, early caducous; calyx 7–10 × 7–9 mm, coriaceous, with scattered appressed trichomes to densely tomentose, lepidote, usually with large external glands arranged in short broad glandular fields, apex denticulate; corolla magenta or lavender, weakly zygomorphic, gradually tubular-infundibular to tubular-campanulate from cylindric base, 4.5–8.8(rarely 10) cm, subcoriaceous to coriaceous, externally tomentose above glabrous base, tube 3.5–7.5 cm with cylindric base 8–20 mm and mouth 13–27 mm wide, internally with tufts of trichomes or minutely papillose at insertion of stamens, otherwise glabrous, lobes (rarely 10)15 22 × 18–22 mm, externally without glandular fields at base, internally lepidote or glabrous; stamens and staminode inserted 7–16 mm from base of corolla tube, shorter stamens with filaments 14–18 mm, included, longer stamens with filaments 21–25 mm, included, all anthers 4–5.5 mm, staminode 3–9 mm; disc 1.5–2 × 4–5 mm; pistil 3.2–3.8 cm, ovary ellipsoid or oblong, 4–5 × 2–3 mm, densely sericeous, style included, pilose at base to lower 3/4,

stigma elliptic to ovate. Capsule elliptic, 8.5–10.5 × 2.7–3.5 cm, 1.5–2 cm in diam., base acute, apex acute to acuminate, valves convex, exocarp separating from adnate mesocarp and endocarp at maturity, minutely puberulent, tomentose or glabrous, surface with ± longitudinal wrinkles, midrib slightly raised apically or flat, wall 2–2.5 mm thick, endocarp densely scabrous. Seeds 1–1.8 × 2.5–3.5 cm, body glabrous or with a few minute trichomes, the wings well demarcated from the seed body, tan, membranous, hyaline.

Figure: Gentry (1982a: fig. 11, note illustration is inaccurate in portraying the inflorescence as a raceme).

Distribution. Specimens have been seen from native populations in Mexico: Chiapas, Oaxaca, Puebla, and Veracruz, and Nicaragua: Estelí and Madriz. Standley (1926) reported *Distictis laxiflora* as also found in Jalisco and Guanajuato, Mexico. No specimens from Jalisco were seen in this study, but it is possible that *Sheldon 52* from Guanajuato, which is cited below, is native. Found in rocky areas or deciduous forests, between 1100 and 2000 m elevation (Fig. 2A).

Phenology. Flowering March to August; fruiting January, May, July, October, and November.

Common names. Vanilla Distictis, Vanilla Trumpet Vine (Menninger et al., 1970); Jeroliz morado, Manto de la Virgen (Standley, 1926).

Uses. Cultivated in tropical and subtropical areas of the world for its beautiful flowers. Menninger et al. (1970) describe the buds as rich purple, the flowers turning lavender and then almost white with the tube streaked with lavender, with "all 3 colors displayed on the vine at the same time in perfect harmony" (Menninger et al., 1970: 108).

De Candolle (1845) published *Pithecoctenium cinereum* DC. with two syntypes, *M. Mairet de Chaudefont s.n.* (G-DC) and *G. Andrieux 221* (G-DC). The type material was examined, the more complete specimen (*M. Mairet de Chaudefont s.n.*, G-DC) selected as lectotype, and the name placed in synonymy of *Distictis laxiflora*. This synonymy follows that of Standley (1926) and Gentry (1974a, 1982a). The type of *P. cinereum* var. *parviflorum* Hochr. was studied and this name placed in synonymy of *D. laxiflora*.

Distictis laxiflora is the only species of *Distictis* known from Central America, and *D. laxiflora* and *D. buccinatoria* are the only species of *Distictis* found in Mexico. They have been hybridized to form the cultivar *Distictis* cv. 'Rivers,' marketed as "Distictis riversii" (see discussion below *D. buccinatoria*). It is not known if they hybridize naturally. The two can be difficult to distinguish when sterile or in flower, but the flowers of *D. buccinatoria* have orangish red corollas, which are usually larger (7.5–12 cm vs. 4.5–8.8 cm), and the longer pair of stamens exserted. The fruits of *D. buccinatoria* are also larger (13–15 × 4–5.5 cm vs. 8.5–10.5 × 2.7–3.5 cm), more oblong, with the apex acute and tip rounded and retuse, and the exocarp does not separate from the adnate mesocarp and endocarp at maturity. Vegetatively, *D. buccinatoria* tends to have larger and more persistent pseudostipules (5–35 × 5–28 mm vs. 3–25 × 3–17 mm), larger leaflets (5–10.5 × 3–6.1 cm vs. 2–8.5 × 1–6 cm) with the lateral veins more prominent abaxially, and trichomes with a golden cast.

Flowering and sterile collections of *Distictis laxiflora* are sometimes confused with *Pithecoctenium crucigerum*. The latter has more sharply angled branchlets, often additionally branched tendrils, the leaflets frequently larger (4.5–17 × 2.2–12 cm vs. 2–8.5 × 1–6 cm), the flowers white, usually strongly curved, and, in Central America, arranged in racemes (vs. panicles).

Distictis laxiflora has sometimes been confused in the horticultural trade with *D. lactiflora* (Menninger et al., 1970), but following Bailey et al. (1976), plants grown under the latter name are probably *D. laxiflora*. *Distictis lactiflora* differs from *D. laxiflora* in its leaflets that are subcoriaceous to coriaceous with very conspicuous, reticulate venation prominent on the adaxial surface, generally smaller (3–5.5 cm), lighter-colored flowers, lepidote (but usually otherwise glabrous) ovaries, and narrow elliptic to fusiform fruits with thin walls with the exocarp not separating from the adnate mesocarp and endocarp at maturity.

Mature fruits of *Distictis laxiflora* generally have both valves convex and the exocarp peeling away from the adnate mesocarp and endocarp; however, one specimen, *C. Conzatti & V. González 308* (TEX) had a curved fruit (one valve convex and the other concave) with an intact exocarp and was probably not fully developed.

Selected specimens examined. MEXICO. **Chiapas:** Amatenango del Valle, *A. S. Ton 2140* (F). **Guanajuato:** Silao, *D. H. Sheldon 52* (GH). **Oaxaca:** s. loc., *G. Andrieux 221* (syntype of *Pithecoctenium cinereum* DC., G-DC not seen, microfiche G-DC 9.195.10); Dtto. Etla, "Barranca Ceniza," al N-NO de El Parian, Mpio. San Jerónimo Sosola, *A. Salinas T. et al. 6888* (MO); Monte Alban, *C. G. Pringle 6724* (MO); Dtto. Teposcolula Mixteca Alta, Río del Oro, Tamazulapan, *A. García M. et al. 301* (MO). **Puebla:** vic. of San Luis Tultitlanapa, near Oaxaca, *C. A. Purpus 3261* (MO). **Veracruz:** Casa Amarilla, carretera las Trancas–Coatepec, Coatepec, *M. G. Zolá B. et al. 335* (MO). NICARAGUA. **Estelí:** Loma Ocotecalzado (Mesas Moropotente), ca. 11 km NE of Hwy. 1 at Estelí, *W. D. Stevens & A. Grijalva 15608*

(MO). **Madriz:** Cerro Quisuca, summit and upper slopes, *W. D. Stevens & P. P. Moreno 22199* (MO).

7. **Distictis occidentalis** A. H. Gentry, Novon 2: 161. 1992. TYPE: Colombia. Santander: 3 km N of Yarima on rd. to Albania SE of Barranca Bermeja, 25 July 1975, *A. H. Gentry & L. Forero 15409* (holotype, MO!; isotype, COL not seen).

Young branchlets sharply hexangular, the angles ribbed and detaching with age, with scattered to dense, golden to ferruginous, dendritic trichomes to 0.1 mm, some furfuraceous and without rachis, sometimes mixed with ones with rachis, the trichome arms usually numerous (rarely less than 4) and equal in length to the internodes; pseudostipules subfoliaceous, orbicular, ovate or broadly oblong, 2–4 × 1.5–4 mm, caducous. Leaves bifoliolate, often with tendrils, tendrils trifid, tips filamentous; petiole 1–4 cm, with trichomes like those of branchlet; petiolule 0.6–4 cm, with trichomes like those of branchlet; leaflets elliptic to oblong-obovate, 5–17.5 × 3–9 cm, membranous to chartaceous, 4 or 5 pairs of lateral veins, tertiary veins mostly parallel and ladder-like, rarely weakly reticulate, midrib and lateral veins flat adaxially and tertiary veins raised or all flat or midrib slightly impressed, midrib and lateral veins prominent abaxially with tertiary veins flat or prominent, adaxial surface usually drying reddish brown (rarely brownish green), usually with some trichomes on midrib (sometimes also on lateral and tertiary veins) furfuraceous-dendritic, scattered to densely lepidote, abaxial surface drying brownish green, with trichomes like those of branchlet usually on midrib and often on lateral veins, sometimes in lateral vein axils to dense over entire surface, then often mixed with trichomes to 0.15 mm with longer arms, densely pellucid-lepidote, occasionally with large glands, base obliquely rounded, or obtuse and then obliquely rounded or subcordate, apex obtuse with tip cuspidate and apiculate, or obtuse or rounded with tip apiculate, sometimes acuminate, the tip often curving. Inflorescence a raceme, peduncle with rachis 5–13 cm with 1 to 16 flowers, peduncle, rachis, and pedicels with trichomes like those of branchlet, bracteoles affixed at base of pedicel, oblong or narrow spatulate, 3–12 × 1.5–2 mm, usually persistent; calyx 5–7 × 5–9 mm, coriaceous, tomentose with furfuraceous-dendritic trichomes strongly appressed, external glandular fields short and broad, apex minutely denticulate (rarely truncate); corolla white with yellow throat, weakly zygomorphic, gradually tubular-infundibular or tubular-campanulate from cylindric base, (rarely 5.5)6.5–9.5 cm, subcoriaceous to membranous, externally with dense minute dendritic trichomes above glabrous base, tube (rarely 4.5)5–7.6 cm with cylindric base 12–24 mm and mouth 17–25 mm wide, internally villose at insertion of stamens, otherwise glabrous, lobes 10–30 × 15–30 mm, externally with linear glandular fields at base, internally with minute, scattered, dendritic trichomes and lepidote; stamens and staminode inserted 15–20 mm from base of corolla tube, shorter stamens with filaments 13–25 mm, included, longer stamens with filaments 22–32 mm, included, all anthers ca. 4 mm, staminode 5–14 mm; disc 1.5–2 × 4–5 mm; pistil 3.1–5 cm, ovary ellipsoid, 3–5 × 2.5–3 mm, densely tomentose with furfuraceous-dendritic trichomes, style included, with dendritic trichomes at base to lower 3/4, stigma orbicular to broadly elliptic. Capsule elliptic, ca. 10 cm, base acute and truncate, apex acuminate, exocarp not separating from the adnate mesocarp and endocarp at maturity, densely furfuraceous-dendritic appressed tomentose, lepidote, with numerous cup-shaped glands especially dense along midrib, wall ca. 2 mm thick. Seeds 12–13 × 15–16 cm, body glabrous, the wings poorly demarcated from the seed body, greatly reduced, brown, coriaceous, opaque.

Figure: Gentry (1992: fig. 1A, B).

Distribution. Northwestern South America from Chocó and Santander, Colombia, to Madre de Dios, Peru, and Santa Cruz, Bolivia. Usually found in seasonally inundated forests or along rivers, between 100 and 300 m elevation (Fig. 1B).

Phenology. Flowering April to July, October. Only one old fruit seen.

No reference to common name or economic use was found for this species.

No whole mature fruits of *Distictis occidentalis* were seen; the description here is based on the broken but apparently mature fruits of *S. F. Smith et al. 1455.*

Distictis occidentalis has seeds with the wings greatly reduced and coriaceous (vs. well developed and membranous-hyaline in most species of *Distictis*), and the body somewhat corky, suggesting that it is water dispersed (vs. wind dispersed), correlating well with its usual habitat of seasonally inundated forests or growth along rivers.

Distictis occidentalis is very similar to *D. granulosa*, and most collections were referred to that species prior to Gentry's 1992 publication of *D. occidentalis*. The two are similar in type and distribution of dendritic trichomes, the shape, size, and persistence of the pseudostipules, the leaflet size, shape, and ladder-like pattern of tertiary veins, the bracteole duration, overall flower size, calyx apex, and presence of corolla glandular fields. *Distictis occidentalis* differs from *D. granulosa* in the color of the corolla lobes (white vs. purple or lavender), leaflet texture (membranous to chartaceous vs. subcoriaceous to co-

riaceous) and lateral veins flat on upper surface versus impressed, shape of calyx glandular fields (short and broad vs. long and narrow), and the seed wings (very reduced, coriaceous-opaque vs. well developed, membranous-hyaline). The two species overlap at Tambopata, Peru, with *D. occidentalis* occurring in swampy areas around lake margins and *D. granulosa* occurring in sandy upland forests (Gentry, 1992).

The only other species of *Distictis* with dendritic trichomes, corolla glandular fields, and white corolla lobes is *D. steyermarkii*. The two species are also similar in pseudostipule persistence, leaflet texture and prominence of venation, and calyx glandular field shape. However, *D. steyermarkii* always has at least some simple trichomes on the leaflet adaxial surfaces and longer dendritic trichomes (0.3–2 mm vs. to 0.1 mm) on vegetative parts, caducous bracteoles, cordate leaflet bases, and well-developed, membranous-hyaline seed wings.

Gentry (1992) suggested two specimens from Peru, *T. B. Croat 18576* and *V. Huashikat 1912* (both in bud), might represent either a new species in *Distictis* or simply a more pubescent form of *D. occidentalis*. I believe that the latter is more likely the case, as *A. H. Gentry et al. 76577* helps to form a continuum between these densely pubescent specimens and the more glabrescent type. He also described the leaflet venation of *T. B. Croat 18576* as being intricately prominulous abaxially. However, many specimens of *D. occidentalis* (such as *O. Haught 2209*) are equally prominulous.

Selected specimens examined. BOLIVIA. **Santa Cruz:** Río Viboro, *F. Billiet & B. Jadin 5986* (BR). COLOMBIA. **Chocó:** Trail from Unguia to Cerro Mali, lowest slopes of Serrania del Darien, *A. H. Gentry & S. Mori 13737* (MO). **Santander:** Rd. from Barranca Bermeja to El Centro near airport, *A. H. Gentry & L. Forero 15398* (MO); vic. of Barranca Bermeja (El Centro), *O. Haught 2209* (MO). ECUADOR. **Sucumbíos:** Downstream Río Cuyabeno from Laguna de Cuyabeno to Puerto Bolivar, *J. Brandbyge et al. 30541* (MO, photocopy of AAU at MO). PERU. **Amazonas:** Valle del Río Santiago, ca. 65 km N de Pringlo, Quebrada Caterpiza, *V. Huashikat 1912* (MO). **Loreto:** 7 km SW of Iquitos, *T. B. Croat 18576* (MO); Quebrada Tamshiyacu, *A. H. Gentry et al. 76577* (MO). **Madre de Dios:** Tambopata Province, Cuzco Amazónico, *P. Núñez et al. 10919* (MO); edge of Laguna Cocacocha, Explorer's Inn, near confluence of Río Tambopata and Río La Torre, 39 km SW of Puerto Molodnado, *S. F. Smith et al. 1455* (MO).

8. Distictis pulverulenta (Sandwith) A. H. Gentry, Ann. Missouri Bot. Gard. 63: 77. 1976. *Distictella pulverulenta* Sandwith, Brittonia 3: 91. 1938. TYPE: Brazil. Amazonas: Basin of Rio Solimoes, Municipality of São Paulo de Olivença, basin of Creek Belem, Oct.–Dec. 1936, *B. A. Krukoff 8685* (holotype, NY not seen, digitized image!; isotypes, BM not seen, BR not seen, F not seen, GH not seen, K not seen, MO!, U not seen).

Young branchlets moderately to sharply hexangular, the angles ribbed (sometimes inconspicuously) and sometimes detaching with age, densely covered with minute, strongly appressed golden, simple trichomes; pseudostipules absent or rarely present, not foliaceous, oblong, ca. 3 × 1.5 mm, caducous. Leaves bifoliolate, occasionally with tendrils, tendrils trifid, tips sometimes disk-like; petiole 1.5–5.5 cm with minute strongly appressed trichomes; petiolule 0.7–2.4 cm with minute strongly appressed trichomes; leaflets elliptic, sometimes asymmetric, 5–14.5 × 3–10.4 cm, subcoriaceous to membranous, 3 to 5(rarely 6) pairs of lateral veins, tertiary veins weakly reticulate to loosely ladder-like, midrib and lateral veins impressed adaxially with tertiary veins prominent or flat or all nearly flat, midrib, lateral, and tertiary veins prominent abaxially or midrib and lateral veins prominent and tertiary veins flat, adaxial surface drying brownish or yellowish green, glabrous or minutely puberulent on main veins, abaxial surface drying greenish gray, usually densely furfuraceous-tomentose, ± canescent, with main veins glabrescent, occasionally surface glabrescent, conspicuous clusters of large glands present in the lower lateral vein axils, base rounded, subcordate, or obtuse, often obliquely, apex obtuse to acute with tip cuspidate. Inflorescence a panicle, peduncle with rachis 6–16 cm with numerous flowers, peduncle, rachis, and pedicels densely covered with minute appressed trichomes, bracteoles spatulate, ca. 10 × 3 mm, early caducous; calyx 9–15 × (rarely 6–)8–10 mm, coriaceous, pubescent with minute appressed trichomes, external glandular fields narrow and oblong to short and broad, apex truncate; corolla tube light purple, lobes dark red- or brown-purple, weakly zygomorphic, tubular-infundibular from cylindric base, (rarely 5)5.6–6.7 cm, membranous to subcoriaceous with base coriaceous, externally tomentose above glabrous base, tube (rarely 4)5–6 cm with cylindric base 8–12 mm and mouth 12–19 mm wide, internally tomentose and lepidote, villose along ridge of insertion of stamens, lobes 10–15 × 10–17 mm, externally without glandular fields at base, internally tomentose and lepidote; stamens and staminode inserted (rarely 8)10–11 mm from base of corolla tube, shorter stamens with filaments 15–19(rarely 21) mm, included, longer stamens with filaments 22–27 mm, included, all anthers 3.5–4 mm, staminode 3–6 mm; disc ca. 1 × 4 mm; pistil 4.2–4.8(rarely 5) cm, ovary ovoid or elliptic, 3–4.5 × 2–2.3 mm, densely covered with minute appressed trichomes, style included, tomentose to apex, stigma rhombic. Capsule elliptic, 7–13.5

× 3–3.6 cm, 0.8–1 cm in diam., base acute and truncate, apex acute, one valve convex and the other concave causing the fruit to be curved, exocarp not separating from the adnate mesocarp and endocarp at maturity, tomentose with minute trichomes, surface smooth, midrib slightly impressed, wall 1.5–2 mm thick. Seeds 1.3–1.8 × 5–7 cm, body glabrous or slightly papillose, the wings poorly demarcated from the seed body, yellowish apically to dark brown at base, membranous, hyaline apically to opaque at base.

Figure: Lohmann & Hopkins (1999: pp. 609, 619, 620).

Distribution. Northeastern Peru, Amazonian Brazil, southern Colombia (A. H. Gentry, pers. comm.), southern Venezuela, Guyana, Suriname (L. Lohmann, pers. comm.), and French Guiana. Found in non-inundated forests on white sand (Gentry, 1979a), frequently in disturbed areas, between 120 and 900 m elevation (Fig. 2A).

Phenology. Flowering January, May to July, November, and December; fruiting December.

No reference to common name or economic use was found for this species.

Distictis pulverulenta was described in the closely allied genus *Distictella* by Sandwith (1938), who emphasized its foliar pubescence and curved fruit in selecting generic placement. Gentry (1976) transferred *Distictella pulverulenta* to *Distictis* based on its hexangular, ribbed branchlets and straight, chartaceous to subcoriaceous, purple corollas all of which contrast with *Distictella*, which has terete branchlets and curved, coriaceous, white corollas. However, *Distictis pulverulenta* is an oddity in the genus *Distictis* based on several vegetative, floral, and fruiting characteristics that are common in *Distictella* but otherwise unknown in *Distictis*: the many-flowered, compoundly branched paniele, the pubescent and lepidote inner surface of the corolla tube, and the curved fruits. In addition, the seeds are very similar to those of *Distictella elongata*, with wings poorly demarcated from seed body, yellow and hyaline at the tips and brown and opaque at the base, with the same irregular ridges and papillae as found on the body. The generic placement of *Distictis pulverulenta* is uncertain, but, pending further study, it is here retained in *Distictis*, following Gentry (1976, 1982b).

Selected specimens examined. BRAZIL. **Amazonas:** Rio Tiqué, entre Fátima e Pari, *J. M. Pires & N. T. Silva 8002* (photocopy of IAN at MO); rd. to Praia Dourado after turnoff from paved rd., near Manaus, *A. H. Gentry & J. Ramos 13297* (MO). FRENCH GUIANA. St. Laurent piste d'Apatou, *C. Feuillet 1457* (MO). GUYANA. Akarai Mtns., height of land betw. drainage of Rio Mapuera (Trombetas tributary) and Shodikar Creek (Essequibo tributary), *A. C. Smith 2979* (MO). PERU. **Loreto:** Province Requena, Genaro Herrera, Río Ucayali below Requena, *A. H. Gentry et al. 21308* (MO); Maynas, Dtto. Iquitos, carretera de Peña Negra a 7 km de Quisto Cocha, *M. Rimachi Y. 5675* (MO); Maynas, Dtto. Iquitos, carretera de Peña Negra a 3 km de Quisto Cocha, *M. Rimachi Y. 7543* (MO). VENEZUELA. **Amazonas:** 4 km E of San Carlos de Río Negro, *R. Liesner 3648* (MO); carretera San Carlos–Solano, 2–6 km SO de Solano, *G. Morillo et al. 3972* (MO).

9. Distictis scabriuscula (Mart. ex DC.) A. H. Gentry, Ann. Missouri Bot. Gard. 61: 499. 1974.

Pithecoctenium scabriusculum Mart. ex DC. in A. DC., Prodr. 9: 197. 1845. TYPE: Brazil. Rio Paraíba, 1826, *Prince Vidensis (Wied-Neuwied) s.n.* (holotype, BR "Herb. Martius"!)

Pithecoctenium scabriusculum var. *macranthum* Bureau & K. Schum. in Mart., Fl. Bras. 8(2): 171. 1896, var. "*macrantha*." Syn. nov. TYPE: Brazil. With inaccurate locality information, (Bureau & K. Schumann, 1896), *A. Glaziou 7776* (holotype, P not seen; isotype, C!, photo F neg. 22129!).

Young branchlets sharply hexangular, the angles ribbed and detaching with age, with scattered to dense, golden to ferruginous, dendritic trichomes to 0.3 mm, some furfuraceous and without rachis mixed with ones with rachis, trichome arms numerous, the arms and internodes equally small; pseudostipules absent. Leaves bifoliolate, frequently with tendrils, tendrils trifid, tips filamentous; petiole 0.6–3 cm, with trichomes like those of branchlet, especially dense adaxially; petiolule 0.3–1.3 cm, with trichomes like those of branchlet, especially dense adaxially; leaflets elliptic, 4–10.2 × 2–6.2 cm, membranous to chartaceous, 3 or 4 pairs of lateral veins, tertiary veins mostly parallel and ladder-like, rarely weakly reticulate, veins all flat adaxially, or slightly impressed, prominent abaxially or with tertiary veins flat, adaxial surface drying reddish brown, with trichomes on midrib like those of branchlet or with scattered dendritic trichomes on surface, sometimes scattered lepidote, abaxial surface drying light brown-green, with trichomes like those of branchlet dense and covering entire surface, or only on the midrib, or trichomes with longer arms and internodes on surface with trichomes on midrib like those of branchlet, scattered pellucid-lepidote, occasionally with large glands, base obliquely obtuse to rounded (rarely subcordate), acute or obtuse with tip cuspidate and apiculate, often curving. Inflorescence a raceme, peduncle with rachis 1.5–4.5 cm with 1 to 4 flowers, peduncle, rachis, and pedicels with dense trichomes like those of branchlet, bracteoles affixed at base of pedicel, linear or awl-like, 6–11 × 0.5–1 mm, caducous; calyx 1.5–7(rarely 9) × 6–7 mm, subcoriaceous, tomentose with dense trichomes like those of branchlet, external glandular fields narrow and

oblong, apex denticulate; corolla with tube yellow and lobes purple to purple-red, weakly zygomorphic, gradually tubular-infundibular or tubular-campanulate from cylindric base, 5.5–6.5 cm, subcoriaceous to membranous, externally with dense minute dendritic trichomes above glabrous base, tube 4.2–4.8 cm with cylindric base 9–10 mm and mouth 13–15 mm wide, internally villose at insertion of stamens, otherwise glabrous, lobes 10–17 × 14–15 mm, externally with linear glandular fields at base or glands somewhat scattered, internally lepidote with few dendritic trichomes; stamens and staminode inserted 11–15 mm from base of corolla tube, shorter stamens with filaments 13–18 mm, included, longer stamens with filaments 19–22(rarely 24) mm, included, all anthers 3.5–4.2 mm, staminode 4–5 mm; disc ca. 2 × 5 mm; pistil 3.2–4 cm, ovary ovoid, ca. 5 × 3.5 mm, densely tomentose with dendritic trichomes like those of branchlet, style included, with dendritic trichomes at base to lower 3/4, stigma obovate. Capsule broadly elliptic, ca. 4 × 2.8 cm, ca. 0.3 cm in diam., base acute and truncate, apex acute and apiculate, valves compressed, exocarp not separating from the adnate mesocarp and endocarp at maturity, tomentose with dense dendritic trichomes, glands numerous, especially apically, midrib flat, wall ca. 1 mm thick. Seeds ca. 17 mm, body puberulent, the wings well demarcated from the seed body, tawny, membranous, hyaline.

No figures found in the literature for this species.

Distribution. Central-eastern Brazil, from Pernambuco to Espírito Santo and west to Serra do Espinhaço of Minas Gerais. Growing in disturbed areas at 950 m (only one collection seen with elevation indicated; Fig. 2B).

Phenology. Flowering February and March. Fruiting February.

No reference to common name or economic use was found for this species.

Type material of *Pithecoctenium scabriusculum* var. *macranthum* Bureau & K. Schum. was studied and not found to be distinct enough from typical material of *P. scabriusculum* Mart. ex DC. to warrant varietal recognition, and this name is placed in synonymy of *Distictis scabriuscula*.

Only one fruit of *Distictis scabriuscula* was seen, and it had retained only one seed with wings torn.

Distictis scabriuscula is similar to *D. frutescens* and *D. granulosa* in having purple corolla lobes, glandular fields on the corollas, and dendritic trichomes. It differs from *D. frutescens* primarily in the absence of pseudostipules, which are large, foliaceous, and persistent in *D. frutescens*, and in having bracteoles caducous (vs. persistent) and narrower (6–11 × 0.5–1 mm vs. 13–22 × 2–8 mm). In addition, the calyx denticules of *D. frutescens* are somewhat more distinct than those of *D. scabriuscula* and the calyx glandular fields somewhat broader. *Distictis scabriuscula* differs from *D. granulosa* in having leaflets membranous to chartaceous versus coriaceous, elliptic versus often obovate and smaller (4–10.2 × 2–6.2 cm vs. 7–19 × 3.5–10.5 cm) with the midrib and lateral veins flat adaxially. The bracteoles of *D. scabriuscula* are caducous and longer and relatively narrower than those of *D. granulosa* (1.5–2 × 1 mm), and the trichomes are larger (to 0.3 mm vs. to 0.15 mm).

Gentry (1974a) placed *Distictis scabriuscula* closest to *D. steyermarkii*. While most collections of *D. steyermarkii* have longer trichomes (0.7–2 mm) with fewer and longer arms, a few specimens have the longest trichomes only 0.3 mm long with numerous, small arms and very small internodes, and are indistinguishable from the longer trichomes of *D. scabriuscula*. However, there are always at least a few simple trichomes on the adaxial surfaces of the leaflets of *D. steyermarkii* (not found in *D. scabriuscula*), and *D. scabriuscula* has some furfuraceous-dendritic trichomes on its vegetative parts, which are absent on vegetative parts of *D. steyermarkii*. In addition, *D. steyermarkii* has subfoliaceous pseudostipules (3–8 × 1.5–5 mm), cordate leaflet bases, calyces with the trichomes minute and strongly appressed and with short and broad glandular fields, and white corolla lobes, while *D. scabriuscula* has the pseudostipules absent, leaflet bases obliquely obtuse to rounded (rarely subcordate), calyces with trichomes longer and spreading and narrow and oblong glandular fields, and purple to purple-red corolla lobes.

Selected specimens examined. BRAZIL. **Bahia:** Rodovia, BR 5, 16 km S of Euriápolis, *R. P. Belém & R. S. Pinheiro 2676* (MO). **Espírito Santo:** Santa Teresa, *W. Boone 8* (MO). **Minas Gerais:** Serra do Espinhaço, SE drainage of Pico de Itambé, about 5 km NW of Santo Antônio de Itambé, *W. R. Anderson et al. 35669* (MO, NY). **Pernambuco:** Paulista, Km 14 da BR 101 ao lado do Posto Esso, *E. Tenório 66/158* (INPA photocopy at MO).

10. Distictis staminea (Lam.) A. H. Gentry, Ann. Missouri Bot. Gard. 61: 497. 1974. *Bignonia staminea* Lam., Encycl. 1: 421. 1785. *Wunschmannia staminea* (Lam.) Urb., Symb. Antill. 5: 494. 1908. TYPE: Dominican Republic. Santo Domingo, t. 56, f. 2, Plum. in Burm., Pl. Amer. 1756 (lectotype, designated by de Candolle, 1845: 161, tab. 56, f. 2, Burm., 1756!; original Plumier drawing, P not seen).

Young branchlets sharply hexangular, the angles ribbed and sometimes detaching with age, densely puberulent with simple trichomes; pseudostipules subfoliaceous, elliptic, ca. 5 × 2 mm, early caducous. Leaves bifoliolate, sometimes with tendrils, tendrils

trifid, tips sometimes disk-like; petiole 0.6–3 cm, puberulent or pilose adaxially; petiolule 0.4–1.5 cm, puberulent or pilose adaxially; leaflets oblong or ovate, 2.4–6 × 1.1–4 cm, subcoriaceous, 3 or 4 pairs of lateral veins, tertiary veins reticulate, midrib, lateral, and tertiary veins conspicuously prominent adaxially, midrib and lateral veins prominent abaxially, drying dark brown-green, adaxial surface minutely puberulent along veins, densely pellucid-lepidote, abaxial surface pilose along main veins, densely pellucid-lepidote, sometimes with large glands scattered near base, base obliquely truncate, apex acute or obtuse with tip retuse. Inflorescence a raceme or racemose panicle, peduncle with rachis 10–20 cm with 4 to 14 flowers, peduncle, rachis, and pedicels puberulent and lepidote, bracteole affixed at base of pedicel, linear, ca. 3 × 0.5 mm, early caducous; calyx 5–8 × 6–8 mm, subcoriaceous, lepidote and with scattered, small trichomes, external glandular fields poorly defined, apex denticulate; corolla orangish yellow to reddish orange, strongly bilabiate with the lower lip reflexed with 3 equal lobes and the upper lip erect and bilobate, narrowly tubular-infundibular from cylindric base, 5.2–6 cm, chartaceous, externally tomentose and lepidote above glabrous base, tube 4–4.5 cm with cylindric base 7–12 mm and mouth 9–11 mm wide, internally villose at insertion of stamens, otherwise glabrous, lower lobes ca. 10 × 8 mm and upper lip ca. 10 × 9 mm, externally without glandular fields at base, internally tomentose and lepidote; stamens and staminode inserted 10–13 mm from base of corolla tube, shorter stamens with filaments ca. 35 mm, included, longer stamens with filaments 39–45 mm, exserted beyond mouth of corolla tube by 10–12 mm, all anthers ca. 4 mm, staminode 8–10 mm; disc 2–3 × 4 mm; pistil longer than 5.5 cm, ovary ovoid or oblong, ca. 4 × 2.5 mm, densely lepidote with scattered trichomes at base, style exserted, glabrous, stigma oblong. Capsule oval.

Figure: Burman (1756: t. 56, f. 2).

Distribution. Endemic to Hispaniola. Growing in semi-arid forest, between 80 and 500 m elevation (Fig. 1A).

Phenology. Flowering January, March, and May; fruiting material not seen.

No reference to common name or economic use was found for this species.

Lamarck's original description (1783) was based on Plumier drawing "5" and the reproduction of the Plumier plate in Burman (1756: t. 56, f. 2), the latter selected by de Candolle (1845) as the lectotype. The plate in Burman (1756), cited by de Candolle (1845), is odd in having simple tendrils from the leaf nodes and compound leaves that terminate in a flower. Following Gentry (1974a) and Urban (1908), these errors are not present in the original Plumier plate at P.

Much of the above description is from Urban (1908) and Liogier (1995b).

Distictis staminea is unique in the genus *Distictis* in having a strongly bilabiate corolla. The corolla of *D. staminea*, which is narrow, orange, with erect bilobed upper lip and spreading 3-lobed lower lip, is reminiscent of the corolla of the monotypic genus *Dolichandra* Cham. of southern South America. The latter differs in its spathaceous calyx and claw-like tendrils. Both are apparently hummingbird pollinated. *Distictis staminea* is similar vegetatively to *D. lactiflora*, the latter differing in the glabrous undersurface of the leaflets, its white, slightly zygomorphic corollas, and included stamens.

Specimens examined. HAITI. Gonaïves ad Grande, *W. Buch 352* (MO); vic. of Port de Paix, *E. C. Leonard & G. M. Leonard 12329* (K not seen, photocopy at MO).

11. **Distictis steyermarkii** A. H. Gentry, Ann. Missouri Bot. Gard. 61: 499. 1974. TYPE: Venezuela. Distrito Federal: betw. Osma and Todasana, 100 m, 24 Nov. 1971, *J. A. Steyermark & C. B. Carias 105274* (holotype, MO!; isotype, VEN not seen).

Young branchlets sharply hexangular, the angles ribbed and detaching, pubescent with scattered to dense, yellow dendritic and/or simple trichomes, longest trichomes (rarely 0.3–)0.7–2 mm, all the dendritic trichomes with rachis, usually with 2 to 4 arms and internodes usually long, rarely with numerous arms, and arms and internodes short; pseudostipules subfoliaceous, lanceolate, 3–8 × 1.5–5 mm, caducous. Leaves bifoliolate, often with tendrils, tendrils trifid, tips filamentous; petiole 1–4.5 cm, with trichomes like those of branchlet; petiolule 0.6–2.5 cm, with trichomes like those of branchlet; leaflets ovate or oblong, 4–15 × 2.5–9 cm, membranous or chartaceous, 3 or 4 pairs of lateral veins, tertiary veins mostly weakly reticulate, rarely ± parallel and ladder-like, midrib and lateral veins flat or impressed adaxially and tertiary veins raised or flat, midrib, lateral, and tertiary veins prominent abaxially, usually drying olive or yellowish green, adaxial surface with scattered simple trichomes (rarely 0.1–)0.7–2 mm, often mixed with dendritic trichomes, usually with 2 to 4 arms (rarely numerous arms), scattered to densely lepidote, abaxial surface with dendritic trichomes, the longest (0.3–)0.7–1 mm with 2 to 4 arms, rarely only to 0.15 mm and with numerous small, closely compressed arms, sometimes mixed with simple trichomes, densely pellucid-

lepidote, occasionally with large glands, base obliquely or symmetrically cordate (rarely subcordate), apex obtuse with tip cuspidate. Inflorescence a raceme, peduncle with rachis 3–11 cm with 4 to 10 flowers, peduncle, rachis, and pedicels pubescent with trichomes furfuraceous-dendritic (rarely some of the trichomes dendritic with rachis), bracteoles affixed at base of pedicel, linear, 3–10 × 0.7–2 mm, caducous; calyx 4–8 × 6–8 mm, coriaceous, tomentose with furfuraceous-dendritic trichomes strongly appressed, external glandular fields short and broad, apex truncate (rarely minutely denticulate); corolla white with yellow throat, weakly zygomorphic, gradually tubular-campanulate from cylindric base, 5.8–7.5 cm, membranous, externally with dense minute dendritic trichomes above glabrous base, tube 4.3–6 cm with cylindric base 10–15 mm and mouth 10–22 mm wide, internally villose at insertion of stamens, otherwise glabrous or lepidote, lobes 10–20 × 11–20 mm. externally with linear-glandular fields at base, internally with scattered minute dendritic trichomes and lepidote; stamens and staminode inserted 10–14 mm from base of corolla tube, shorter stamens with filaments 17–20 mm, included, longer stamens with filaments 23–29 mm, included, all anthers 4–5 mm, staminode 5–6 mm; disc ca. 1 × 2–3 mm; pistil 3.5–4.9 cm, ovary ellipsoid, 3–5 × ca. 2 mm, densely tomentose with furfuraceous-dendritic trichomes, style included, with dendritic trichomes nearly to apex, stigma broadly elliptic. Capsule elliptic, 7.5–11 × 2.2–3.5 cm, ca. 1.5 cm in diam., base acute and truncate, apex acute, both valves convex, exocarp not separating from the adnate mesocarp and endocarp at maturity, tomentose with minute dendritic trichomes, numerous cup-shaped glands especially dense along midrib, midrib slightly impressed, wall ca. 1–2 mm thick. Seeds 1–1.5 × 3–3.9 cm, body minutely puberulous, the wings well demarcated from the seed body, pale brown, membranous-hyaline.

Figure: Gentry (1982b: fig. 16).

Distribution. Northwestern Venezuela, from Miranda to Zulia and Táchira and adjacent Colombia: Cesar and Norte de Santander (A. H. Gentry, pers. comm.). Found in deciduous forests and disturbed areas, between sea level and 1400 m elevation (Fig. 2A).

Phenology. Flowering August, October, November, February, and March; fruiting November.

No reference to common name or economic use was found for this species.

Distictis steyermarkii differs from the other species of *Distictis* with dendroid trichomes and corolla glandular fields in having at least some simple trichomes present on the adaxial leaf surface, and the trichomes, especially those of petiole and petiolule, are usually long (rarely 0.3–)0.7–2 mm. It is most similar to *D. occidentalis* by virtue of having pseudostipules similar in size and persistence, leaflet texture membranous to chartaceous, calyx glandular field short and broad, and corollas white with yellow throats. However, the two differ not only in form and length of vegetative trichomes (to 0.15 mm in *D. occidentalis*) but also in the bracteoles, which are generally persistent in *D. occidentalis*, leaflet bases, which vary from obliquely rounded to subcordate in *D. occidentalis*, and seed wings, which are greatly reduced, coriaceous, and opaque in *D. occidentalis*.

Gentry (1974a) placed *Distictis steyermarkii* closest to *D. scabriuscula*, which differs in having furfuraceous-dendritic trichomes on the branchlets, petioles, petiolules, and inflorescence (vs. dendritic trichomes with rachis and simple trichomes), pseudostipules absent (vs. present, but caducous), leaflets without simple trichomes (vs. present on adaxial surfaces), with bases usually obliquely obtuse to rounded (vs. usually cordate), calyx glandular fields narrow and oblong (vs. short and broad), and corolla lobes purple to purple-red (vs. white).

Selected specimens examined. COLOMBIA. **Cesar:** Rincon Hondo, Magdalena Valley, *C. Allen 424* (MO). VENEZUELA. **Distrito Federal:** Dept. Vargas, 0.6 km W of Oritapo, 7 km E of Osma, *J. A. Steyermark & V. Carreño Espinoza 106874* (MO); E of Los Caracas, vic. of Osma, *A. H. Gentry & P. Berry 14758* (MO). **Miranda:** 36 km W of Boca de Uchire, *A. H. Gentry et al. 10375* (MO). **Táchira:** Pan-American Hwy., 12 km NE of La Fria, *A. H. Gentry et al. 11049* (MO). **Zulia:** Dept. Colón, alrededores de Casigua El Cubo, sector Las Cruces, 1–2 km al S de Casigua rumbo a El Carmelo, *G. S. Bunting & F. Arboleda L. 8071* (MO); Dept. Colón, carretera Casigua–Palmira–Encontrados, entre Casigua y km 15 de la vía, *G. S. Bunting & M. Fucci 9831* (MO).

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Appendix 1. Index to Exsiccatae.

Collections are alphabetical by collector name. After the collector's number, the number in parentheses corresponds to the species number in the List of Species. Type collections appear in boldface. All collections cited here were examined by this author as part of this study.

List of Species

1. *Distictis buccinatoria* (DC.) A. H. Gentry
2. *Distictis frutescens* (DC.) A. Pool
3. *Distictis gnaphalantha* (A. Rich.) Greenm.
4. *Distictis granulosa* Bureau & K. Schum.
5. *Distictis lactiflora* (Vahl) DC.
6. *Distictis laxiflora* (DC.) Greenm.
7. *Distictis occidentalis* A. H. Gentry
8. *Distictis pulverulenta* (Sandwith) A. H. Gentry
9. *Distictis scabriuscula* (Mart. ex DC.) A. H. Gentry
10. *Distictis staminea* (Lam.) A. H. Gentry
11. *Distictis steyermarkii* A. H. Gentry

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Ekman 827 (3), *7324* (3), *15549* (5), *16637* (5), *17703* (5), *18535* (5), *19185* (3), *H996* (5), *H9459* (5), *H9625* (5).

Feuillet 1457 (8), *1458* (4), *2122* (8). *Fischer s.n.* (6).

Galeotti 7060A (6), *7060I* (6). *Garcia M. et al. 301* (6). *Gentry 9361* (7), *11963* (1), *11984* (1), *12793* (4), *12842* (4), *12848* (4), *50829* (3), *51020* (3). *Gentry & Berry 14521* (4), *14542* (4), *14758* (11). *Gentry & Daly 18737* (7). *Gentry & Estensoro 70258* (4), *70258B* (4). *Gentry & E. Forero 7241B* (7). *Gentry & L. Forero 15398* (7), ***15409* (7)**. *Gentry & Mejía 50685* (5), *50736* (5), *50814* (5). *Gentry & Mori 13737* (7). *Gentry & Núñez 69320* (7), *69427* (4), *69550* (7). *Gentry & Puig-Ross 14294* (11). *Gentry & Ramos 13297* (8). *Gentry & Stein 47160* (4). *Gentry & Zardini 50467* (5). *Gentry & Zanoni 50546* (5), *50642* (5). *Gentry et al. 10375* (11), *10633* (4), *10657* (4), *10684* (4), *11049* (11), *19658* (7), *20999* (8), *21308* (8), *31739* (8), *51393* (4), *56274* (8), *56526* (8), *66090* (5), *68968* (7), *76577* (7), *76710* (4), *76932* (4). *Glaziou* ***7776* (9)**, ***8812* (2)**. *González V. et al. 3667* (1). *Greenway 10900* (1).

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NICORAEPOA (POACEAE, POEAE), A NEW SOUTH AMERICAN GENUS BASED ON *POA* SUBG. *ANDINAE*, AND EMENDATION OF *POA* SECT. *PARODIOCHLOA* OF THE SUB-ANTARCTIC ISLANDS[1]

Robert J. Soreng[2] *and Lynn J. Gillespie*[3]

Abstract

Poa subg. *Andinae* Nicora (Poaceae, tribe Poeae) is raised to the rank of genus, here renamed as *Nicoraepoa* Soreng & L. J. Gillespie. Six specific and one infraspecific combinations are made: *N. andina* (Trin.) Soreng & L. J. Gillespie, *N. chonotica* (Phil.) Soreng & L. J. Gillespie, *N. erinacea* (Speg.) Soreng & L. J. Gillespie, *N. pugionifolia* (Speg.) Soreng & L. J. Gillespie, *N. robusta* (Steud.) Soreng & L. J. Gillespie, *N. subenervis* (Hack.) Soreng & L. J. Gillespie, and *N. subenervis* subsp. *spegazziniana* (Nicora) Soreng & L. J. Gillespie. Lectotypes are designated for *N. pugionifolia* and *N. subenervis*. Taxonomy and nomenclature of similar Southern Hemisphere island species of *Poa* L. and other related genera are discussed. *Poa* sect. *Parodiochloa* (C. E. Hubb.) Soreng is emended to include, in addition to *P. flabellata* (Lam.) Raspail, *P. cookii* (Hook. f.) Hook. f., *P. foliosa* (Hook. f.) Hook. f., *P. hamiltoni* Kirk, *P. ramosissima* Hook. f., and *P. tennantiana* Petrie. The one species of *Tzvelevia* E. B. Alexeev is reunited with *Poa*, and the genus is transferred to *Poa* sect. *Tzvelevia* (E. B. Alexeev) Soreng & L. J. Gillespie. Morphological and anatomical characteristics of *Nicoraepoa*, *Poa* sect. *Parodiochloa*, and similar genera in Poinae are compared. Leaf blade morphology and anatomy were found to be particularly useful in characterizing *Nicoraepoa* and related taxa. A key is provided to distinguish *Nicoraepoa*, *Poa* sect. *Parodiochloa*, and morphologically similar perennial genera of Poinae.

Key words: *Austrofestuca*, *Bellardiochloa*, *Festucella*, *Hookerochloa*, *Nicoraepoa*, *Parodiochloa*, *Poa*, Poaceae, Poinae, *Tzvelevia*.

Poa subgen. *Andinae* Nicora (Poaceae, tribe Poeae) was described for a group of robust, tough, gynodioecious species from Patagonia having stolons or rhizomes, long staminodes (1–3 mm) in pistillate plants, smooth or scabrous lemmas with glabrous or pilose keel and nerves, and a callus that is glabrous or with rigid hairs to 3 mm long (Nicora, 1977). Some species also have awns at the lemma apex (e.g., *P. andina* Trin.; Nicora, 1978; Soreng, pers. obs.). Nicora (1978) included six species in *Poa* subgen. *Andinae* (*P. andina* Trin., *P. borchersii* Phil. (= *P. chonotica* Phil.), *P. erinacea* Speg., *P. pugionifolia* Speg., *P. robusta* Steud., *P. stepparia* Nicora), but retained the morphologically similar *P. flabellata* (Lam.) Raspail and *P. subenervis* Hack. in subgenus *Poa* as the latter two are perfect-flowered. The lead author thought that the subgenus probably needed to be removed from *Poa* L. since he first saw material of the group in the early 1980s. With new material from collection trips to Chile in 2000–2001 (by R. and N. Soreng) and Argentina in 2001 (by P. M. Peterson, R. J. Soreng, N. Refulio, and M. Belgrano), the opportunity to study types and other specimens, and the continued study of the large genus *Poa* (ca. 500+ species), the morphological and anatomical distinctness of *Poa* subgen. *Andinae* has become clearer, supporting the conclusion that most species of the subgenus and *P. subenervis* (placed in *Poa* subgen. *Poa* by Nicora, 1978) do not belong in *Poa*. However, there was always uncertainty about whether the group repre-

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sented a new genus or could logically be placed within an existing genus in subtribe Poinae and, if it was distinct, how best to delimit it.

Several genera of Poinae had to be considered in the decision on how to treat *Poa* subgen. *Andinae*. Understanding the relationship of *P. flabellata* (sect. *Parodiochloa* (C. E. Hubb.) Soreng) to *P.* subgen. *Andinae* species was critical in deciding what genus name to use, because that species had been transferred to a new genus, *Parodiochloa* C. E. Hubb. (Hubbard, 1981). These groups share morphological features such as thick-ribbed leaf blade adaxial surfaces and awned lemma apices. Other genera morphologically similar to *Poa* subgen. *Andinae*, or otherwise putatively allied to *Poa* (Alexeev, 1976, 1985; García-González, 1983; Clayton & Renvoize, 1986), are *Austrofestuca* (Tzvelev) E. B. Alexeev s. str., *Bellardiochloa* Chiov., *Festucella* E. B. Alexeev, *Hookerochloa* E. B. Alexeev, and *Tzvelevia* E. B. Alexeev. As presently circumscribed, these genera include a total of only nine or 10 species that all, except for *Bellardiochloa* of southeastern Europe and the Middle East, occur in the Southern Hemisphere, in Australia, New Zealand, Tierra del Fuego, and on sub-Antarctic islands. All of the above genera have been controversial, and, except for *Bellardiochloa*, all their species were originally described in *Festuca* L. or other genera other than *Poa* and have since been placed in the above new genera and, in most cases, also in *Poa* (Hooker, 1844–1860; Alexeev, 1976, 1985; Clayton & Renvoize, 1986; Tzvelev, 1989; Jacobs, 1990; Watson & Dallwitz, 1992; Sharp & Simon, 2002; Soreng et al., 2003a; Gillespie & Soreng, 2005). *Poa flabellata*, for example, had also been placed in *Dactylis* L., *Festuca*, *Parodiochloa*, and *Sesleria* Scop.

Recent chloroplast DNA (cpDNA) analyses placed species of *Poa* subgen. *Andinae* outside of *Poa* in a clade with (Soreng et al., 2007) or near (Gillespie & Soreng, 2005; Gillespie et al., 2007) the panarctic genus *Arctagrostis* Griseb., while ITS data support a clade of these two taxa plus two Australian genera, *Festucella* and *Hookerochloa* (Gillespie et al., in prep.). Hunter et al. (2004) provided the first molecular evidence that the latter two genera, placed in *Austrofestuca* by Jacobs (1990), were closely related to each other and isolated from *Poa*, and that *Austrofestuca* s. str. could be included within *Poa*. The latter result was not unexpected as *A. littoralis* (Labill.) E. B. Alexeev is morphologically quite similar to *P. macrantha* Vasey. Additional molecular studies (Gillespie & Soreng, 2005; Gillespie et al., 2007; Soreng et al., 2007) and morphological comparisons have corroborated this result, and *Austrofestuca* is now treated as a monotypic section of *Poa* (Gillespie & Soreng, 2005). *Parodiochloa*, which included the single species *Parodiochloa flabellata* (Lam.) C. E. Hubb., was also shown to belong within *Poa* and is now placed in its own section within *Poa* (Soreng et al, 2003a; Gillespie & Soreng, 2005; Gillespie et al., 2007). Resolution of the generic taxonomy problem remained incomplete, however, as there is also a small group of sub-Antarctic island species of *Poa* that were thought to be closely related to *Parodiochloa* (Edgar, 1986), and thus potentially related to *Poa* subgen. *Andinae*, and no molecular data were available for these or for the monotypic sub-Antarctic island genus *Tzvelevia*. Another genus of subtribe Poinae, the Eurasian genus *Bellardiochloa*, was previously placed in *Poa* (Clayton & Renvoize, 1986) or was thought to be closely related (García-González, 1983). Molecular analyses have consistently shown that *Bellardiochloa* does not belong within *Poa* (Soreng et al., 1990; Davis et al., 1993; Soreng & Davis, 2000), but it has only recently been demonstrated that this genus is not a sister to or direct ancestor of either *Poa* or *Poa* subgen. *Andinae* (Soreng et al., 2007; Gillespie et al., 2007).

The present paper outlines the nomenclature and taxonomy of a new genus established here to accommodate *Poa* subgen. *Andinae*. We discuss how it may be distinguished from *Poa* and other genera of Poeae subtribe Poinae, especially those genera thought to be most closely related. A revised taxonomy is also presented for the morphologically similar *Poa* sect. *Parodiochloa* and *Tzvelevia*, which here is reduced to a section in *Poa*. New data on leaf blade structural characters are presented and discussed here. Gillespie et al. (in prep.) will provide details and discussion of our new molecular analyses, including both plastid and nuclear DNA sequences, which support the taxonomic conclusions of this paper.

Materials and Methods

Specimens, including types, were borrowed from the following herbaria: BAA, CONC, K, MO, SGO, and SI. Additional material was reviewed at BAB, HIP, LE, LP, SI, US, and W. Specimens at US were used for anatomical work. Additional representative specimens are cited as vouchers for geographic ranges of the species cited beyond those ranges vouchered in selected published works. Vouchers for taxa depicted in Figures 1–3 are given in the figure legends. Leaf surfaces were examined and photographed using a Philips XL30 ESEM LaB6 microscope (Eindhoven, The Netherlands) at the National Museum of Natural History, Smithsonian Institution. Sections of blades were taken from about 1 cm above the collar of lower culm leaves of herbarium specimens, attached to

stubs, and sputter coated with 12–18 nm of 60:40 gold:palladium alloy in a Cressington Scientific 108 Auto/SE (Watford, U.K.). Both leaf blade abaxial and adaxial surfaces were examined. Leaf trans-sectional anatomy was examined by cutting rough sections with a handheld razor blade and observing these under a dissecting microscope (50×) or a compound microscope (100×).

Results and Discussion

Nicoraepoa—a new southern South American genus

Poa andina, *P. chonotica*, *P. erinacea*, *P. pugionifolia*, *P. robusta*, and *P. subenervis* are wetland plants that combine a set of characters that are either absent, rare, or unusual in other species of *Poa*. These characters include awned lemmas, a crown of callus hairs, basal sheaths becoming coarsely fibrous in age, ciliate ligules, corrugated adaxial leaf blade surfaces, pungent leaf blade tips, and stout rhizomes. None of the species have all these features, but all share several of them (Table 1). The above species are hereafter referred to as *Nicoraepoa* Soreng & L. J. Gillespie species. All *Nicoraepoa* species occur in Patagonia and most are restricted to this area. Only *N. subenervis* subsp. *spegazziniana* (Nicora) Soreng & L. J. Gillespie extends slightly northward in the moderately high parts of the Andes to 32°04′S (San Juan province, Argentina). One species, *N. robusta* (Steud.) Soreng & L. J. Gillespie, reaches the Falkland/Malvinas Islands. Nicora (1978) provides an excellent key and illustrations to all these species in *Flora Patagónica*.

Characters distinguishing *Nicoraepoa* from *Poa*

SEM anatomical investigations have advanced our understanding of *Poa* and surrounding genera and bolstered our opinion that *Poa* subgen. *Andinae* deserves generic status. *Nicoraepoa* differ from most *Poa* and related genera in the leaf blade adaxial surface (Figs. 1–3). *Nicoraepoa* leaf blade adaxial surfaces are covered by multiple, regularly spaced, tall, costal ridges that are broadly rounded to square- or rectangle-shaped on top and with intercostal valleys between the ridges that are deep, narrow, and smooth or sparsely to moderately densely scabrous (Fig. 1A–F). *Poa* (*Poa* type) leaf blades typically have relatively flat adaxial surfaces with only two central grooves, one on either side of the midrib (Fig. 3E, F) (Lewton-Brain, 1904; Vukolov, 1929; Prat, 1932; Metcalfe, 1960; Hernández Cardona, 1978; García-González, 1983). Costal ridges are present in *Poa*, but, with few exceptions, these are of low relief, narrow, and with intercostal zones several times wider (Fig. 3E, F). Corrugated adaxial leaf blade surfaces like or approaching those in *Nicoraepoa* are rare in *Poa* but do occur in the following species: *P. cookii* (Hook. f.) Hook. f., *P. flabellata*, *P. foliosa* (Hook. f.) Hook. f., *P. hamiltonii* Kirk, *P. macrantha*, and *P. tennantiana* Petrie (Figs. 2E, F, 3A–C, H). *Nicoraepoa* species lack papillae on both long cells and short cells (Fig. 1A–F), whereas *Poa* species with costal/intercostal topography like or approaching *Nicoraepoa* (Figs. 2A–F, 3A–D) commonly have papillate long cells (Figs. 2E, F, 3A–C).

Vicoraepoa and *Poa* also differ in leaf blade internal anatomy and blade apex, ligule, and rhizome morphology, in addition to leaf adaxial-surface morphology. In leaf blade transections of *Nicoraepoa*, the adaxial ridges are underlain by sclerenchyma caps, or by prominent "T"- or "I"-shaped sclerenchyma girders connecting the adaxial surface through the primary vascular bundles to the abaxial surface (Tables 1, 2). Such broad caps or flared-topped girders have not previously been observed in *Poa* to our knowledge (Lewton-Brain, 1904; Vukolov, 1929; Prat, 1932; Metcalfe, 1960; Hernández Cardona, 1978; García-González, 1983). The apices of the blades in *Nicoraepoa* are pungent and are not or only obscurely prow-shaped, whereas in *Poa* they are commonly narrowly to broadly prow-shaped and are rarely pungent. In *Poa*, bulliform cells (visible in cross sections) occur only in the central two grooves; this is typical of Poinae genera with only two central grooves, including *Hookerochloa* and *Tzvelevia*. For those species with multiple broad ridges, we have seen bulliform cells only in the grooves immediately adjacent to the midrib, but more detailed anatomical cross-sectional work is needed to confirm this.

Nicoraepoa ligule apices (Figs. 4C, I, Q, 5B, F, K) are truncate (sometimes slightly raised on the outer margins; Fig. 5B), and ciliolate to ciliate (hairs 0.15–0.6 mm long), whereas in *Poa* ligule apices vary in shape from truncate to acuminate, while the margins are commonly smooth or scabrid, or infrequently ciliolate. *Nicoraepoa* species produce thick rhizomes that are mostly unidirectional. Many species of *Poa* have rhizomes, but apart from the few species of *Poa* subgen. *Arctopoa* (Griseb.) Prob., these rhizomes generally are more slender and sometimes have multidirectional shoots (Serebryakova, 1965).

Floret characters distinguishing *Nicoraepoa* and *Poa* include lemma shape, awn presence, and callus hair distribution and form. *Nicoraepoa* have lemma apices that are sharply pointed, and some are terminated by distinct slender scabrous awns up to 5 mm long. *Poa* typically have slightly blunt lemma apices that lack awns (except in *P. flabellata*). In

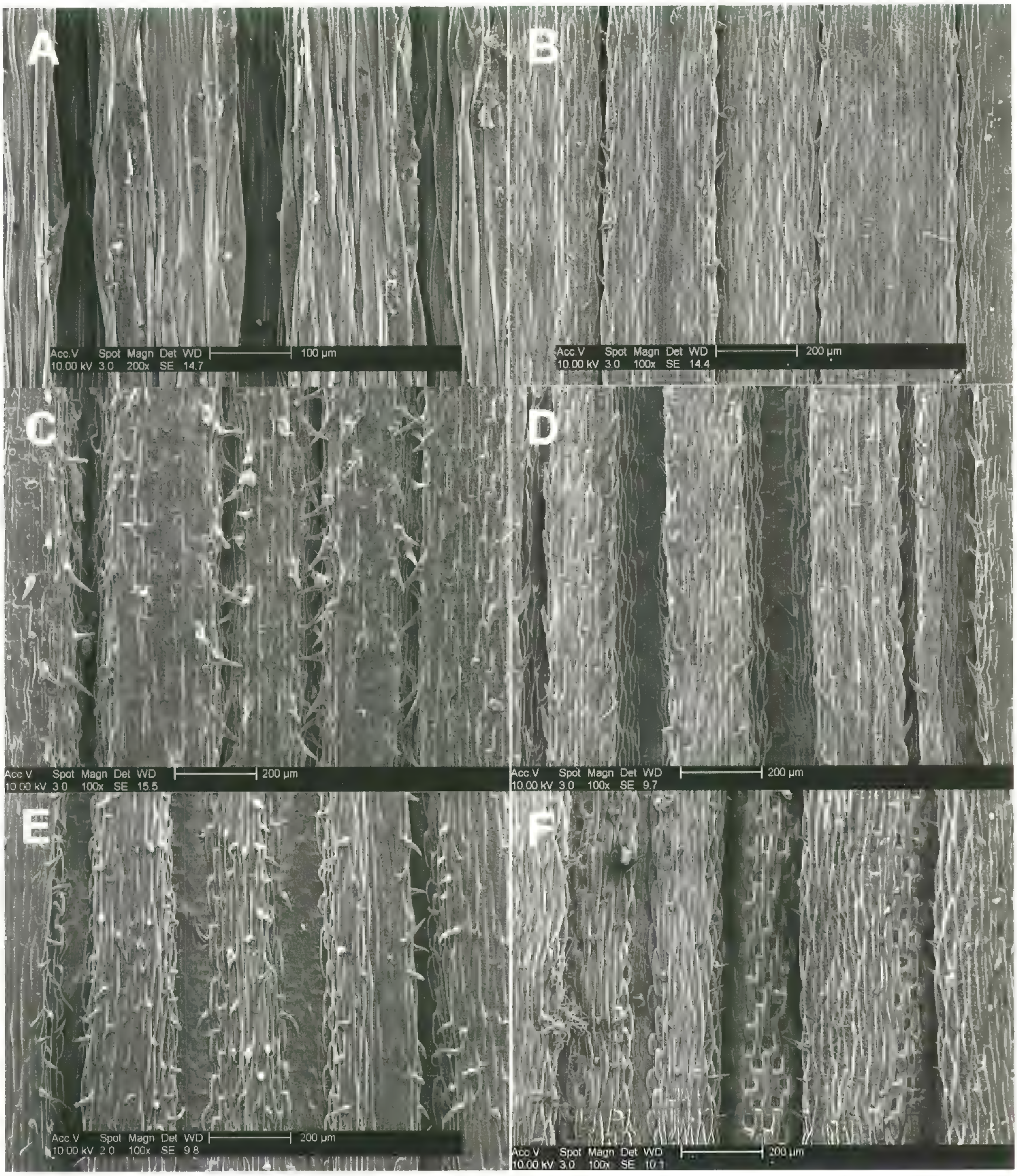

Figure 1. SEM photographs of adaxial surfaces of leaf blades of *Nicoraepoa* Soreng & L. J. Gillespie. —A. *N. andina* (Trin.) Soreng & L. J. Gillespie. Chile, Bío-Bío, *R. J. & N. L. Soreng 7182* (US). —B. *N. chonotica* (Phil.) Soreng & L. J. Gillespie. Chile, Los Lagos, *R. J. & N. L. Soreng 7236* (US). —C. *N. pugionifolia* (Speg.) Soreng & L. J. Gillespie. Chile, Magallanes, *R. J. & N. L. Soreng 7336* (US). —D. *N. robusta* (Steud.) Soreng & L. J. Gillespie. Chile, Magallanes, *R. J. & N. L. Soreng 7359* (US). —E. *N. subenervis* (Hack.) Soreng & L. J. Gillespie subsp. *subenervis*. Chile, Magallanes, *R. J. & N. L. Soreng 7331* (US). —F. *N. subenervis* subsp. *spegazziniana* (Nicora) Soreng & L. J. Gillespie. Chile, Metropolitana, *R. J. & N. L. Soreng 7167* (US).

Nicoraepoa, the calluses of the lemmas are glabrous (*P. robusta* only) or have a row of slightly curved hairs distributed around the base of the lemma. This row of callus hairs is called a crown, as distinct from a beard (commonly used to describe elongated calluses with short stiff hairs across and down the surface) or a web (an isolated dorsal tuft of hairs known only in the genus *Poa*). A few *Poa* species have callus hairs in a crown (e.g., *P. eminens* J. Presl [subgenus *Arctopoa*], *P. secunda* J. Presl [section *Secundae* V. L. Marsh ex Soreng], and *P. obvallata* Steud. [section *Dioicopoa* E. Desv.]), but *Poa* species typically have a single dorsal tuft (a "web") of woolly, or plicate (in section *Dioicopoa*), hairs. Some species of *Poa* with webs

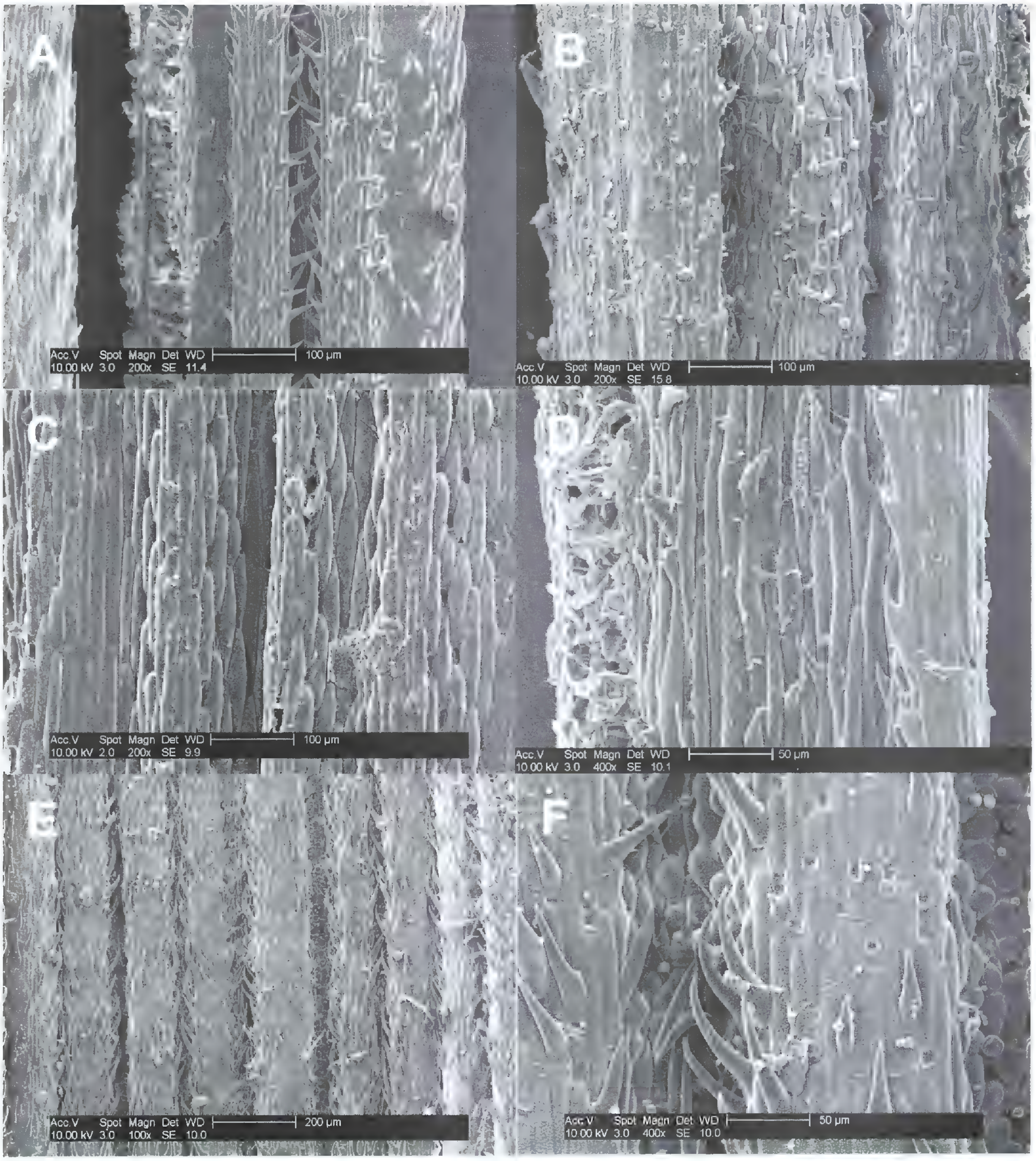

Figure 2. SEM photographs of adaxial surfaces of leaf blades of genera of subtribe Poinae. —A. *Bellardiochloa variegata* (Lam.) Kerguélen. Left side abaxial epidermis and margin. Greece, Peloponnese, *R. J. & N. L. Soreng & L. J. Gillespie 7519* (US). —B. *Festucella eriopoda* (Vickery) E. B. Alexeev. Left side abaxial epidermis and margin. Australia, New South Wales, *E. N. McKie 7449* (US 2010746). —C. *Hookerochloa hookeriana* (F. Muell. ex Hook. f.) E. B. Alexeev. Australia, Tasmania, *R. A. Black* (US 1937360). —D. *Poa kerguelensis* (Hook. f.) Steud. (sect. *Tzvelevia* (E. B. Alexeev) Soreng & L. J. Gillespie). Right side abaxial epidermis and margin. Indian Ocean, Kerguelen Island, Wilkes Expedition (US 653666). —E. *Poa flabellata* (Lam.) Raspail (sect. *Parodiochloa* (C. E. Hubb.) Soreng). South Georgia, *Admr. Berggren* (US 2042368). —F. *Poa flabellata*. Close-up of costal intercostal region with papillate cells in intercostal region. South Georgia, *Admr. Berggren* (US 2042368).

have additional tufts of hairs emerging from the callus at the base of the marginal veins of their lemmas.

Reproductive characteristics also distinguish *Nicoraepoa* from most *Poa* (Nicora, 1977, 1978). All *Nicoraepoa* species, except *N. subenervis*, are gynodioecious with staminodes 1–3 mm long in pistillate flowers (Fig. 4G, N). In contrast, *Poa* species are mostly perfect-flowered or, when diclinous, they generally have pistillate flowers with rudimentary staminodes 0.1–0.2 mm long (Nicora, 1978; Anton & Connor, 1995; Soreng & Keil, 2003; for exceptions, see Edgar & Connor, 2000; Negritto & Anton, 2000).

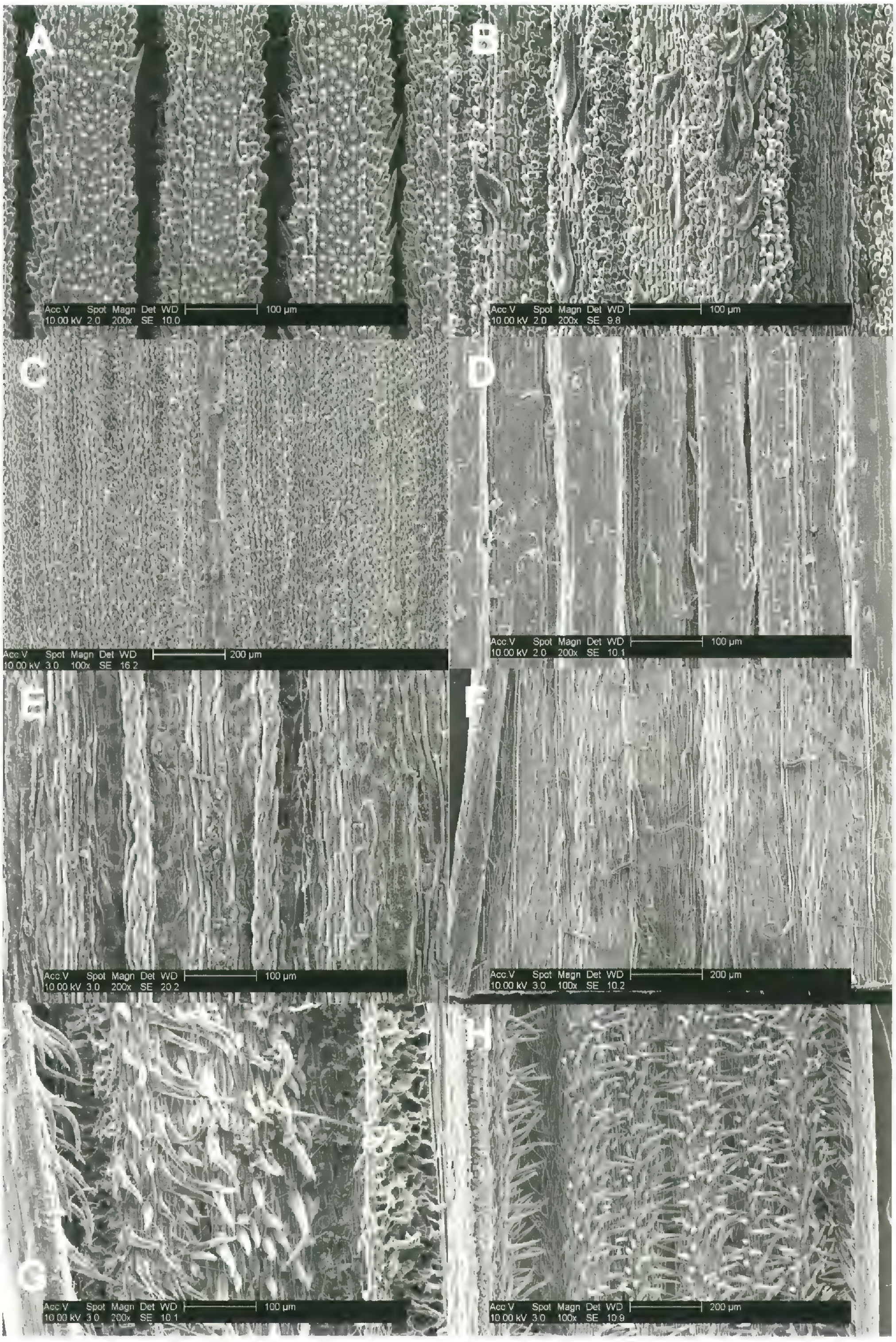

Figure 3. SEM photographs of adaxial surfaces of leaf blades in *Poa* L. A–D. *Poa*, sect. *Parodiochloa* (C. E. Hubb.) Soreng. —A. *P. cookii* (Hook. f.) Hook f. Indian Ocean, Marion and Prince Edward Islands, *B. J. Huntly* (US 3000037). —B. *P. foliosa* (Hook. f.) Hook f. New Zealand, The Snares, *F. L. S. Demedin s.n.* [hb. D. Petrie] (US 947494). —C. *P. ramosissima* Hook. f. Midrib just left of center. New Zealand, Campbell's Island, *J. H. Sorensen* (US 2184040). —D. *P. tennantiana* Petrie. New Zealand, The Snares, *B. C. Aston* [hb. 31857] (US 2044107 (ex hb. Cheeseman)). —E. *P. alpina* L. (sect. *Alpinae* Stapf).

ADDITIONAL TAXONOMIC AND MORPHOLOGICAL CONSIDERATIONS CONCERNING PARTICULAR SPECIES

Nicora (1977, 1978) excluded *Poa subenervis* from her subgenus *Andinae* because it has perfect flowers. However, we consider it to belong in *Nicoraepoa* because it shares the stout rhizomatous habit, the adaxial leaf surface anatomy of broad ridges and narrow valleys (Fig. 1E, F), sharply pointed leaf apices, ciliate ligules, and calluses with a crown of hairs (Figs. 4E, L, 5D, M, N). DNA analyses from both nuclear and plastid genomes support this relationship (Gillespie et al., 2007; Gillespie et al., in prep.).

Poa stepparia, although placed in *Poa* subgen. *Andinae* by Nicora (1977, 1978), needs further study before it can be placed in the new genus. Leaf blade adaxial surfaces of this species are of the *Poa* type with ridges that are only indistinctly raised. The leaf blade apices are pungent, the lemmas lack awns, calluses are glabrous or the marginal vein and keel hairs run into the callus, and the ligules are slightly raised on the sides with somewhat asperous margins. This species was thought to be related to *P. acinaciphylla* E. Desv. (Nicora, 1978), and Soreng et al. (2003a) placed both these species and *P. planifolia* Kuntze in *Poa* sect. *Acutifoliae* Pilg. ex Potztal. We concur with M. Negritto (pers. comm.) that *P. plicata* Hack. (type and sole species of *Poa* sect. *Plicatae* Pilg. ex Potztal) also belongs to this section and place *Poa* sect. *Plicatae* in synonymy of *Poa* sect. *Acutifoliae* (see Taxonomic Treatment section). Analyses of chloroplast and nuclear ITS data confirm *P. acinaciphylla* as a member of *Poa* s. str. (Gillespie & Soreng, 2005; Gillespie et al., 2007; Gillespie et al., unpublished data). *Poa stepparia*, however, is only known from the type and two other collections (Nicora, 1978), and no material has been available for molecular or SEM analyses; its relationships and breeding system need confirmation.

RELATIONSHIPS OF *NICORAEPOA*

Initial cpDNA results from *trnT-trnF* (Gillespie & Soreng, 2005; Gillespie et al., 2007), plus new nuclear ribosomal ITS data (Gillespie et al., in prep.), show that the *Nicoraepoa* taxa align in a clade outside of *Poa* and can logically be placed in a separate genus as they do not fall within any other genus. Species sampled for DNA to date are *N. andina*, *N. chonotica*, *N. pugionifolia*, *N. robusta*, and *N. subenervis*. Preliminary results resolve *Arctagrostis*, *Festucella*, and *Hookerochloa* as the closest genera to *Nicoraepoa*. The relationship of this set of genera to other Poinae genera, *Arctophila* (Rupr.) Rupr. ex Andersson, *Bellardiochloa*, *Dupontia* R. Br., and *Poa* (including *Anthochloa* Nees & Meyen, *Austrofestuca*, *Dissanthelium* Trin., *Eremopoa* Roshev., *Neuropoa* Clayton), is unsettled. Molecular relationships among genera of subtribe Poinae and intermixed genera of Poeae subtribes Alopecurinae and Miliinae are complex and are not yet well resolved or understood. Further sampling is needed before firm conclusions about exact relationships can be drawn (Soreng & Davis, 2000; Gillespie & Soreng, 2005; Soreng et al., 2007; Gillespie et al., 2007; Gillespie et al., unpublished data).

Genera of Poinae thought to be related to *Nicoraepoa* and *Poa* are discussed below, and their morphological characteristics, including leaf blade adaxial surface (Figs. 2A–F, 3G), are compared. *Festucella* and *Hookerochloa* are two closely related genera of Australia and New Zealand. Although sometimes treated within *Austrofestuca* (Clayton & Renvoize, 1986; Jacobs, 1990; Sharp & Simon, 2002), recent molecular data support their separate recognition (Hunter et al., 2004; Gillespie et al., 2007). With *Nicoraepoa*, they share wetland habitats, fibrous basal sheaths, awned lemmas, and a short crown of hairs around the callus, but their leaf blades are *Poa*-like in adaxial anatomy (Fig. 2B, C). The relationships among the Arctic genus *Arctagrostis* and the Southern Hemisphere genera *Nicoraepoa*, *Festucella*, and *Hookerochloa* detected in our molecular analyses need further investigation. ITS data place *Arctagrostis* plus *Nicoraepoa* as sister to *Festuca* plus *Hookerochloa*, but relationships detected with our plastid data are less well resolved at this point. The one-flowered spikelets of *Arctagrostis* are impossible to confuse with the several-flowered spikelets of the other three genera. Its broad, flat leaf blades with narrow, low costal ridges and broad intercostal zones (not shown) do not resemble the blades of these genera.

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Close-up of central region of blade with rows of bulliform cells (protruding here in the intercostal regions) flanking either side of the midvein. Canada, British Columbia, *R. J. & J. E. F. Soreng 5991* (US). —F. *P. pratensis* L. (sect. *Poa*). Narrow costal regions with elongated hairs characteristic of the species. Canada, British Columbia, *R. J. & J. E. F. Soreng 5987* (US). —G. *P. billardierei* St.-Yves (sect. *Austrofestuca* (Tzvelev) Soreng & L. J. Gillespie). New Zealand, South Island, *V. D. Zotov* (US 2010241). —H. *P. macrantha* Vasey (sect. *Madropoa* Soreng). Showing the presence of a more corrugated costal–intercostal surface, a rare exception in *Poa* (and *P. macrantha*). U.S.A., Oregon, *R. J. Soreng 2959* (US).

Table 1. Morphological comparison of species of *Nicoraepoa* and morphologically similar Poinae genera (except *Poa*, which is given in Table 2).

	Arctophila fulva Nyman	*Bellardiochloa variegata*	*Dupontia fisheri* R. Br.	*Festucella eriopoda*	*Hookerochloa hookeriana*	*Nicoraepoa andina*	*Nicoraepoa chonotica*	*Nicoraepoa erinacea*	*Nicoraepoa pugionifolia*	*Nicoraepoa robusta*	*Nicoraepoa subenervis*
Habitat	arctic tundra and northern boreal wetlands, shallow ponds, marshy meadows, swamps, and quiet streams	grassy alpine meadows, commonly over sedimentary rocks	arctic tundra wetlands, mainly coastal, marshy meadows, marginally sub-saline tolerant	montane forest openings near wetlands	montane wetlands	alpine and subalpine riparian and seeps	montane to sea level wetlands, marshes, and lake shores	inland sub-saline flats	inland seasonal wetlands, and sub-saline flats and seeps	coastal saline to sub-saline marshes and seeps	montane sub-saline wetlands, about seeps and springs
Habit, culm height	rhizomatous, 0.05–1 m	densely tufted, 0.09–0.6 m	rhizomatous, 0.05–0.8 m	low tufts, culms 0.50–1.6 m	large tufts, culms 0.55–1.85 m	tufts from a stout little branched rhizome, to 1 m	tufts from a stout little branched rhizome, to 2 m	low tufts from stout little branched rhizomes, 0.07–0.15 cm	low tufts from stout little branched rhizomes, 0.2–0.3 m	tufts from a stout little branched rhizome, to 1 m	low tufts from stout little branched rhizomes, 0.15–0.6 m
Basal sheaths	chartaceous, smooth, retrorsely strigose, to finely fibrous with cross-vein connections	chartaceous, weakly fibrous	chartaceous, or a bit fibrous, smooth, glabrous to sparingly retrorsely strigose	fibrous	fibrous	fibrous, smooth	fibrous, smooth	somewhat fibrous, smooth	somewhat fibrous, smooth	fibrous, smooth	somewhat fibrous, smooth
Blade adaxial lateral surface topography	rounded tall to medium height ridges 0.25–1× wider than valleys (similar to abaxial surface)	medium height, rounded ridges 1× wider than valleys, valleys open	flat or faintly undulating	flat or faintly undulating, obscure ridges	flat	tall flat-topped ridges 2–4× wider than valleys, valleys closed	tall flat-topped ridges 3–5× wider than valleys, valleys closed	flat	tall flat-topped ridges 2–3× wider than valleys, valleys closed	tall flat-topped ridges 2–3× wider than valleys, valleys closed	middle height rounded ridges 1–2× wider than valleys, valleys open or closed

Table 1. Continued.

	Arctophila fulva Nyman	*Bellardiochloa variegata*	*Dupontia fisheri* R. Br.	*Festucella eriopoda*	*Hookerochloa hookeriana*	*Nicoraepoa andina*	*Nicoraepoa chonotica*	*Nicoraepoa erinacea*	*Nicoraepoa pugionifolia*	*Nicoraepoa robusta*	*Nicoraepoa subenervis*
Blade lateral adaxial surface vestiture	smooth, glabrous	ridge tops and sides coarsely hispidulous	smooth or moderately scabrous, glabrous	short stout hooks common to very common, glabrous	short stout hooks common over VBs, glabrous	ridge sides with slender hooks occasional, glabrous	ridge sides and sometimes tops with minute hooks common, glabrous	fairly densely hispidulous-strigulose	ridge sides with hooks common, ridge tops with short hooks occasional, glabrous	ridge sides and sometimes tops with short hooks common, glabrous	ridge sides with short hooks occasional to common, glabrous
Girdering	I-shaped, thick, present in primaries, keel, and most secondaries, keel girdered, abaxially thick, round in cross section	I-shaped, thin, or more often discontinuous, with narrow caps over VBs and low mounds below, keel VB continuous to lower side and with an isolated cap above	I-shaped, thin, usually continuous, but quite narrow and sometimes broken just above the VBs (lacunae below adaxial epidermis)	discontinuous, low basal mounds only	I-shaped primaries, secondaries discontinuous to adaxial side	T-shaped primaries and keel	T-shaped primaries and keel	N/K	caps below ridges, no girders, narrow isolated U below keel	T-shaped primaries and keel	I-shaped, narrow, present in primaries, keel, and some secondaries
Abaxial sclerenchyma along epidermis	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous?	discontinuous	discontinuous	discontinuous
Vascular bundles appearing at adaxial surface	10 to 17/side, alternating primaries and slightly smaller secondaries	3 to 4/side	5 to 8/side	2/side	6/side (12/side abaxially)	10 to 11/side	24/side	2/side	5/side	7/side	5 to 12/side
Ligule texture, color	membranous, white or bronzy	membranous, white	membranous, bronzy	chartaceous, white	chartaceous, white	membranous-chartaceous, white	membranous-chartaceous, white	membranous-chartaceous, white	membranous-chartaceous, white	membranous-chartaceous, white	membranous, white
Ligule length	2–6(8) mm	2–7 mm	1–2.5 mm	1-3 mm, 2–7 mm on basal shoots	2–6 mm	1–3 mm	1–3 mm	0.5 mm	0.5–2.5 mm	0.8–1.5 mm	1–1.5 mm

Table 1. Continued.

	Arctophila fulva Nyman	*Bellardiochloa variegata*	*Dupontia fisheri* R. Br.	*Festucella eriopoda*	*Hookerochloa hookeriana*	*Vicoraepoa andina*	*Vicoraepoa chonotica*	*Vicoraepoa erinacea*	*Vicoraepoa pugionifolia*	*Vicoraepoa robusta*	*Vicoraepoa subenervis*
Ligule abaxial surface	smooth, glabrous	smooth or somewhat scabrous, glabrous	smooth, glabrous	with short hooks common, glabrous	with short hooks frequent, glabrous	hispidulous	densely covered with short thick appressed hairs to hispidulous	smooth or with sparse short stiff hairs	densely covered with hooks to short thick hairs	densely covered with short thick appressed hairs to hispidulous	densely hispidulous
Collar margins	smooth, glabrous	smooth or scabrous	smooth, glabrous	scabrous	smooth or sparsely scabrid	hispidulous	hispidulous	hispidulous	hispidulous	hispidulous	ciliate
Inflorescence shape, length	open lax, 5–30 cm	contracted or loosely contracted, 5–25 cm	contracted or open panicle, 3–18 cm	open, 4.5–40 cm	open, 18–24 cm	contracted, interrupted, 5–20 cm	loosely contracted and interrupted to open lax, 5–30 cm	contracted, narrow, 2.5–4.5 cm	contracted, narrow, 3–6 cm	contracted, or in viviparous forms loosely contracted	contracted narrow panicle or sometimes lax loose
Panicle branches	smooth	branches densely scabrous	smooth	densely scabrous angled	densely scabrous angled	smooth	smooth	densely ciliate scabrid angled	moderately scabrid angled	smooth	smooth
Lemma awn	absent or occasionally with a cusp to 0.8 mm long	slender cusp or awn 0.5–1.5 mm long, from the apex or a short notch	absent or occasionally with cusp or thin awn up to 0.6 mm long	apiculate to slender awn (0–)0.5–1.5(–2) mm long, from a minute notch	slender awn 0.5–2(–3) mm long, from a minute notch	short slender awn 0.5–3(–5) mm long	apiculate	apiculate	absent to apiculate	absent to apiculate	apiculate
Callus	with crown of straight hairs around the base, hairs 0.3–0.7 mm long	with crown of hairs 0.3–0.6 mm long	with crown of coarse hairs 0.5–1.2 mm long	with crown of hairs 0.4–0.6 mm long	with crown of hairs 0.3–0.6 mm long	with crown of slender hairs 2–3 mm long	with crown of slender hairs 1–2.5 mm long	with crown of hairs 0.2–0.3 mm long	glabrous	glabrous	with crown of hairs 0.4–1.5 mm long
Lodicule length, shape, and margin	with a lateral lobe	irregularly dentate or with 1 to 2 slender lobes in addition to main slender lobe, glabrous or ciliate	usually with a lateral lobe	0.5 mm, lobed as in a mitten, or shouldered at midpoint, glabrous	0.8 mm, lobed as in a mitten or shouldered at midpoint, glabrous or with 1 or 2 cilia	0.9–1 mm, lanceolate to irregularly slender lobed, glabrous or with a cilia	1.5 mm, broadly lanceolate glabrous	N/K	0.9 mm, irregularly lobed or shouldered, glabrous	1.7 mm, broadly lanceolate glabrous	0.5–0.8 mm, lanceolate, irregularly toothed, glabrous

Table 1. Continued.

	Arctophila fulva Nyman	*Bellardiochloa variegata*	*Dupontia fisheri* R. Br.	*Festucella eriopoda*	*Hookerochloa hookeriana*	*Nicoraepoa andina*	*Nicoraepoa chonotica*	*Nicoraepoa erinacea*	*Nicoraepoa pugionifolia*	*Nicoraepoa robusta*	*Vicoraepoa subenervis*
Caryopsis shape	fusoid, with a distinct sulcus	fusoid, sub-cylindrical to subtriagonous, sulcus indistinct to moderately distinct	fusoid, subtriangular, with an indistinct sulcus	fusoid, subtriangular, sulcus distinct	fusoid, subtriangular, sulcus distinct	fusoid, subtriangular, sulcus distinct	fusoid, subtriangular, sulcus distinct	N/K	fusoid, subtriangular, sulcus distinct	fusoid, subtriangular, sulcus distinct	fusoid, subtriangular, sulcus distinct
Fruit length, hilum length, hilum shape	1.5–2 mm, 0.25 mm, broadly ovate to punctiform	1.8–2.5 mm, 1/8–1/6 length of grain, ovoid to punctiform	1.5–2.5 mm long, 1/6–1/5 length of grain, broadly ovate	3 mm, 0.6 mm, narrowly elliptical	3.8 mm, 0.8 mm, elliptical	2 mm, 0.4 mm, elliptical	3 mm, 0.8 mm, narrowly elliptical	N/K	3 mm, 0.4 mm, elliptical	3.4 mm, 0.6 mm, narrowly elliptical	2.2 mm, 0.4 mm, elliptical to broadly elliptical
Lipid presence, caryopsis firmness	N/K	present, firm to semi-soft	N/K	present, firm	present, firm	present, firm	present, firm	N/K	present, firm to semi-soft	present, firm	present, firm
Styles, stigma	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose
Chromosomes	$2n = 42, 63$	$2n = 14$	$2n = 42, 44, 88, 132$	N/K	N/K	N/K	N/K	N/K	N/K	N/K	N/K

VB = vascular bundles; N/K = not known.

Table 2. Morphological comparison of species of *Poa* sect. *Parodiochloa* and morphologically similar *Poa* sections.

	Poa (subg. *Arctopoa*) sect. *Aphydris* (Griseb.) Tzvelev	*P.* (subg. & sect. *Arctopoa*) *eminens*	*P.* (sect. *Austrofestuca*) *billardierei*	*P.* (sect. *Parodiochloa*) *cookii*	*P.* (sect. *Parodiochloa*) *flabellata*	*P.* (sect. *Parodiochloa*) *foliosa*	*P.* (sect. *Parodiochloa*) *hamiltonii*	*P.* (sect. *Parodiochloa*) *ramosissima*	*P.* (sect. *Parodiochloa*) *tennantiana*	*P.* (sect. *Tzveleria*) *kerguelensis*	*Poa* (other than those separated)
Habitat	steppe and alpine, riparian and sub-saline seasonally wet flats, sub-saline springs	coastal, wet meadows, rocky beaches, sub-saline	coastal sand dunes and sandy to shingly flats	coastal, among rocks and peat mounds, and on slopes, often associated with penguin colonies	coastal, bluffs, meadows and rocky ledges	coastal slopes, sometimes coastal strand turfy meadows	coastal, among rocks and peat mounds, and on slopes, often associated with penguin colonies	coastal, wet rocks and cliffs, commonly associated with bird colonies	coastal, forest margins, clearings in scrub, and on banks	rocky oceanic island slopes 100–400 m	all cool temperate to arctic habitats, generally intolerant of saline conditions
Habit, culm height	rhizomatous or infrequently stooling, to 1.15 m	rhizomatous, to 1 m	dense tufts from deeply buried culms which may appear like rhizomes, to 0.6 m	robust dense tussocks, to 0.5 m	massive tussocks, to 1 m	massive tussocks with short thick stolons, to 1.5 m	dense tussocks, to 0.5 m	weak culmed, stoloniferous, culms prostrate, distally erect	tufts from stout rhizomes, to 1 m	dense, low tufts, to 0.15 m	perennial or infrequently annual, tufted, rhizomatous, or stooling
Basal sheaths	fibrous and chartaceous, smooth, retrorsely strigose	chartaceous, smooth, retrorsely strigose	fibrous, smooth, glabrous	fibrous, pectinate ciliate between ridges	fibrous, pectinate ciliate between ridges	fibrous, smooth, glabrous	fibrous, smooth, glabrous	papillate, glabrous	fibrous, pectinate ciliate between ridges	finely fibrous, glabrous	not becoming fibrous or rarely becoming fibrous, smooth or finely scabrous particularly along ridges, glabrous or infrequently hispidulous
Blade adaxial lateral surface topography	flat	flat or slightly undulate	flat or faintly undulating	tall flat-topped ridges 3–4× wider than valleys, valleys closed	low to medium height rounded ridges, 1–2× wider than valleys, valleys open (abaxial similarly ribbed)	low flat-topped ridges 1–2× wider than valleys, valleys closed or open and shallow	low flat-topped ridges 1–2× wider than valleys, valleys slightly open	low flat-topped ridges 2–3× wider than valleys, valleys open	low flat-topped ridges 1–2× wider than valleys, valleys slightly open	flat	flat or infrequently ridges slightly raised, ridges 0.2–1× wider than valleys, valleys open

Table 2. Continued.

	Poa (subg. *Arctopoa*) sect. *Aphydris* (Griseb.) Tzvelev	*P.* (subg. & sect. *Arctopoa*) *eminens*	*P.* (sect. *Austrofestuca*) *billardierei*	*P.* (sect. *Parodiochloa*) *cookii*	*P.* (sect. *Parodiochloa*) *flabellata*	*P.* (sect. *Parodiochloa*) *foliosa*	*P.* (sect. *Parodiochloa*) *hamiltonii*	*P.* (sect. *Parodiochloa*) *ramosissima*	*P.* (sect. *Parodiochloa*) *tennantiana*	*P.* (sect. *Tzvelevia*) *kerguelensis*	*Poa* (other than those separated)
Blade lateral adaxial surface vestiture	smooth, glabrous	smooth, glabrous	densely coarsely scabrous to stiffly puberulent	valleys to ridge margins densely papillate to minutely scabrid, sometimes with additional larger slender hooks occasional, glabrous	valleys papillate, ridge sides with slender hooks frequent, glabrous	valleys to ridge margins densely papillate, ridge tops with thick-bodied hooks common, glabrous	valleys to ridge margins densely papillate, glabrous	valleys to ridge margins densely papillate, glabrous	smooth or sparsely papillate, rarely minutely scabrid on ridges, glabrous	short stout hooks frequent, glabrous	smooth or costally scabrid, or infrequently generally scabrid or hispid puberulent or pilulose, rarely papillate
Girdering	I-shaped primaries	I-shaped primaries, narrow I-shaped secondaries, tertiaries without girders	I- or Λ-shaped VB, primaries, and secondaries progressively reduced to margin, narrowest at contact of adaxial epidermis, keel thickest	T-shaped primaries to I-shaped secondaries, bases thick, capitals sometimes discontinuous just above VBs, and in keel, thick in lower keel	I-shaped primaries, thick hourglass shape, secondaries slightly narrower, continuous between VBs in primaries and secondaries, keel isolated U-shape below	I-shaped primaries, thick, secondaries free, continuous between VBs in primaries, secondaries free above and below VBs, keel girdered above with isolated mound below	T-shaped primaries slender, to I-shaped secondaries	I-shaped primaries, continuous between VBs except in keel, keel with isolated mound below	I-shaped primaries, thick, secondaries mostly continuous, keel VBs girdered above with mound connected below	discontinuous, low basal mounds only, isolated keel U capped below	I-shaped or discontinuous, sometimes discontinuous above or below, primaries and secondaries common
Abaxial sclerenchyma along epidermis	discontinuous	discontinuous	continuous, thickest below VBs	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous or rarely continuous between VBs

Table 2. Continued.

	Poa (subg. *Arctopoa*) sect. *Aphydris* (Griseb.) Tzvelev	*P.* (subg. & sect. *Arctopoa*) *eminens*	*P.* (sect. *Austrofestuca*) *billardierei*	*P.* (sect. *Parodiochloa*) *cookii*	*P.* (sect. *Parodiochloa*) *flabellata*	*P.* (sect. *Parodiochloa*) *foliosa*	*P.* (sect. *Parodiochloa*) *hamiltonii*	*P.* (sect. *Parodiochloa*) *ramosissima*	*P.* (sect. *Parodiochloa*) *tennantiana*	*P.* (sect. *Tzvelevia*) *kerguelensis*	*Poa* (other than those separated)
Vascular bundles appearing at adaxial surface	5 to 7/side	8 to 9/side apparent, but 18 to 19/side including tertiaries	5/side	16 to 21/side, primaries mainly	10 to 17/side, primaries mainly	12 to 17/side, primaries mainly	6 to 15(21?)/side, primaries and secondaries	5 to 6/side	15 to 17/side, secondaries frequent	3/side	2 to 11/side
Ligule texture, color	membranous to membranous-chartaceous, off-white to yellowish or brownish	membranous-chartaceous, yellow-cream to brown	chartaceous laterally, membranous-chartaceous centrally, white	chartaceous-membranous, white	chartaceous basally to membranous apically, white	chartaceous, white	membranous-chartaceous, white	membranous, white	chartaceous basally to membranous apically, white	membranous, white	membranous, white or clear
Ligule length	1–4(5.5) mm	1–3.5 mm	0.7–1 mm	4–7.5 mm	6–23 mm	1–3 mm	4–7.5 mm	(1.5)2.5–4 mm	6–16 mm	ca. 3 mm	0.2–18 mm
Ligule abaxial surface	scabrous, glabrous	smooth, glabrous	moderately scabrous, glabrous	smooth, glabrous	with occasional short hooks, glabrous	densely covered with short thick appressed hairs (finely scabrous, f. Edgar, 1985)	smooth, glabrous	densely papillate, glabrous	scabrid to puberulent	with occasional short hooks, glabrous	smooth, scabrous, or short puberulent
Collar margins	smooth, glabrous or ciliate	smooth, glabrous	smooth, glabrous	smooth, glabrous	smooth, glabrous	smooth, glabrous	smooth, glabrous	smooth to papillate	smooth to ciliate	smooth or sparsely scabrid, glabrous	glabrous or infrequently puberulent
Inflorescence shape, length	open or contracted, 5–35 cm	contracted, 8–30 cm	narrowly contracted, (3.5–)6–25 cm	contracted, 5–25 cm	tightly contracted, dense, branches and axis somewhat contorted, 5–20 cm	contracted, fairly dense, 10–20 cm	contracted, 5–25 cm	narrow, loosely contracted, (2)4–5(10) cm	loosely contracted, 9–16 cm	narrowly contracted, 2–4 cm	tightly contracted to wide open and lax

Table 2. Continued.

	Poa (subg. *Arctopoa*) sect. *Aphydris* (Griseb.) Tzvelev	*P.* (subg. & sect. *Arctopoa*) *eminens*	*P.* (sect. *Austrofestuca*) *billardierei*	*P.* (sect. *Parodiochloa*) *cookii*	*P.* (sect. *Parodiochloa*) *flabellata*	*P.* (sect. *Parodiochloa*) *foliosa*	*P.* (sect. *Parodiochloa*) *hamiltonii*	*P.* (sect. *Parodiochloa*) *ramosissima*	*P.* (sect. *Parodiochloa*) *tennantiana*	*P.* (sect. *Tzvelevia*) *kerguelensis*	*Poa* (other than those separated)
Panicle branches	smooth to densely scabrous angled	smooth or sparsely scabrous	smooth below, coarsely spreading scabrous all around distally and on pedicels (rarely sparsely so)	smooth, densely papillate, sometimes coarsely scabrous, the hooks spreading (and then papillae obscure)	smooth	smooth or with a few hooks	smooth, densely papillate	smooth, densely papillate	occasionally smooth, commonly sparsely scabrous above and on pedicels	densely scabrid on and less so between angles	smooth or scabrous (rarely papillate), hooks diffuse or more often in lines above vascular traces, glabrous or rarely hispidulous
Lemma awn	absent	absent	absent	cuspidate	cuspidate to short stout awn	cuspidate	cuspidate	cuspidate	cuspidate	absent or cuspidate	absent or rarely cuspidate
Callus	glabrous, or with 1 to several straight hairs (to 1.5 mm long) dorsally and below the marginal veins	usually with a line of 1–2 mm long slightly sinuous hairs around base of proximal lemmas, sometimes all glabrous	short crown of hairs around the callus, hairs stiff, 0.5–0.8 mm long	glabrous or with a loose crown of coarse slightly sinuous hairs	glabrous	dense tuft of crinkled hairs dorsally, sometimes with a few hairs laterally	glabrous	glabrous or with dorsal tuft of hairs, sometimes with a few hairs laterally, hairs closely sinuous to crinkled	glabrous	glabrous	mostly with a dorsal web, sometimes glabrous, infrequently with a crown of hairs or a diffuse web
Lodicule length, shape, and margin	0.9 mm, deeply to shallowly lobed, glabrous	1.5 mm, obovate, with a small lateral lobe, glabrous	1.25–1.5 mm, with 2 broadly lanceolate lobes, lobes with a few apical short cilia	0.8–0.9 mm, obovate, margin with or without a few cilia along the upper margins	0.6 mm, with 2 lobes, with or without 1 or 2 cilia	0.4–0.7 mm, weakly lobed, glabrous, infrequently or rarely with 1 or 2 cilia	0.9 mm, infrequently or rarely with 1 or 2 cilia	0.3–1 mm, with a lateral tooth, glabrous or infrequently with 1 or 2 cilia	0.5–0.8 mm, N/K, N/K	0.8 mm, lobed or shouldered at midpoint, glabrous	generally lobed as in a mitten, infrequently glabrous or rarely with 1 or 2 cilia in some New Zealand species (f. Edgar, 1885)

Table 2. Continued.

	Poa (subg. *Arctopoa*) sect. *Aphydris* (Griseb.) Tzvelev	*P.* (subg. & sect. *Arctopoa*) *eminens*	*P.* (sect. *Austrofestuca*) *billardierei*	*P.* (sect. *Parodiochloa*) *cookii*	*P.* (sect. *Parodiochloa*) *flabellata*	*P.* (sect. *Parodiochloa*) *foliosa*	*P.* (sect. *Parodiochloa*) *hamiltonii*	*P.* (sect. *Parodiochloa*) *ramosissima*	*P.* (sect. *Parodiochloa*) *tennantiana*	*P.* (sect. *Tzvelevia*) *kerguelensis*	*Poa* (other than those separated)
Caryopsis shape	fusoid, subtriangular, sulcus distinct	fusoid, subtriangular, sulcus distinct	apically blunt, subpentangular, sulcus pronounced	fusoid, nearly cylindrical, sulcus indistinct	fusoid, subtriangular, sulcus distinct	fusoid, nearly round to subtriangular, sulcus indistinct	fusoid, nearly cylindrical, sulcus indistinct	fusoid	fusoid, nearly cylindrical, sulcus indistinct	narrowly fusoid, subtriangular, sulcus distinct	fusoid, subtriangular, or nearly cylindrical, sulcus distinct, or faint
Fruit length, hilum length, hilum shape	2–? mm, 0.4–? mm, elliptical	2.5–4.5 mm, 0.4–0.6 mm, elliptical	2.5–4 mm, ca. 1 mm, elliptical	ca. 2 mm, ca. 0.3 mm, elliptical	2–2.5 mm, ca. 0.4 mm, elliptical	2–2.7 mm, ca. 0.3 mm, broadly elliptical	ca. 1.5 mm, ca. 0.3 mm, elliptical	ca. 1 mm, presumably short < 1/2 length of grain	ca. 1.5 mm, ca. 0.2 mm, elliptical to punctiform	ca. 1.8 mm, ca. 0.25 mm, narrowly elliptical	1–4 mm, 0.2–0.4 mm, punctiform to elliptical
Lipid presence, caryopsis firmness	present, firm	present, firm	present, firm	present, firm	present, firm	present, firm	N/K	N/K	present, firm	present, firm	present, firm
Styles, stigma	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases without a gap between them, slender with only short primary branches	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose	bases with a gap, plumose
Chromosomes	$2n$ = 42, 91, 97	$2n$ = 28, 42, 62	$2n$ = 28	N/K	N/K	$2n$ = 28	N/K	$2n$ = 28	$2n$ = 56	N/K	[illegible]

VB = vascular bundles. N/K = not known.

The single species of *Austrofestuca* s. str. is now treated as *Poa billardierei* St.-Yves (≡ *A. littoralis* (Labill.) E. B. Alexeev) (subg. *Poa* sect. *Austrofestuca* (Tzvelev) Soreng & L. J. Gillespie) based on DNA sequence evidence (Hunter et al., 2004; Gillespie & Soreng, 2005; Gillespie et al., 2007). *Austrofestuca* leaf blades are similar to *P. macrantha* (subg. *Poa* sect. *Madropoa* Soreng) in involution and vestiture (Fig. 3G, H), and both are coastal sand-dune species. Blades of both species have involute margins and are densely covered by prickle hairs ca. 100 μm long, as is typical of *Poa* sect. *Madropoa* and many Australian species of *Poa*. In *P. billardierei*, the leaf blades have only two distinct grooves on the adaxial surface, as is typical of *Poa*, whereas *P. macrantha* can have up to three lateral grooves on each side of the midrib, with low costal ribs up to 2.5× the width of the valleys (a condition not previously reported in any other species of *Poa*). *Poa* sect. *Austrofestuca* is perfect-flowered, however, whereas *Poa* sect. *Madropoa* species are diclinous, often dioecious as in *P. macrantha*.

Little has been published about the single species of *Tzvelevia* (Alexeev, 1985), an endemic of the Kerguelen Islands and Heard Island. The genus was not mentioned by Clayton and Renvoize (1986) or Watson and Dallwitz (1992). The combination of *Poa*-type leaf blade adaxial anatomy (Fig. 2D), keeled, unawned, softly pubescent lemmas, glabrous calluses, and acute, entire ligule margins supports its relationship with *Poa* rather than *Nicoraepoa*. *Tzvelevia* does not have papillae in the adaxial or abaxial leaf blade epidermis but does have sparse short hooks adaxially. Scant sclerenchyma is developed between the vascular bundles and the epidermis, and it appears to be discontinuous, not forming girders. For these reasons, we submerge *Tzvelevia* in *Poa* as a section (see Taxonomic Treatment section).

The four species of alpine southeastern Europe and the Middle East in the genus *Bellardiochloa* (type, *B. violaceae* (Bellardi) Chiov. (= *B. variegata* (Lam.) Kerguélen)) have been placed in (Clayton & Renvoize, 1986) and out of *Poa*, sometimes near *Poa* (Edmondson, 1980), or in a subtribe with *Festuca* rather than with *Poa* (Tzvelev, 1983; Mill, 1985). Molecular analyses have consistently shown that *B. variegata* does not belong within *Poa* (Soreng et al., 1990; Davis et al., 1993; Soreng et al., 2007; Gillespie et al., 2007). It differs from *Poa* in leaf blade anatomy, with multiple tall costal ridges and deep valleys (Fig. 2A; Hernández Cardona, 1978; García-González, 1983), slender leaf blade apices, weakly keeled lemmas with awns, calluses with a crown of hairs, ciliate lodicule margins, sometimes semi-soft endosperm, and a crown of hairs at rachilla joint apices. With *Nicoraepoa*, the genus *Bellardiochloa* shares the deeply corrugated leaf blade adaxial epidermis and short-awned lemmas, but differs in the narrow costal ridges, weakly keeled lemmas, ciliate lodicule margins, and a crown of hairs at rachilla joint apices.

AN EXPANDED SUB-ANTARCTIC *POA* SECT. *PARODIOCHLOA*

Analyses of cpDNA and ITS data place *Parodiochloa flabellata* within *Poa*, above the basal split of the remainder of *Poa* from section *Sylvestres* V. L. Marsh ex Soreng (and, in cpDNA analyses, subgenus *Arctopoa* (Griseb.) Prob.). The species is placed within the subgenus *Ochlopoa* (Asch. & Graebn.) Hyl. clade in cpDNA analyses, and between the subgenus *Ochlopoa* clade and the large higher *Poa* clade including subgenera *Poa*, *Pseudopoa* (K. Koch) Stapf, and *Stenopoa* (Dumort.) Soreng & L. J. Gillespie in ITS analyses (Gillespie & Soreng, 2005; Gillespie et al., 2007; Gillespie et al., in prep.). The single species of *Parodiochloa* was elevated within *Poa* to *Poa* sect. *Parodiochloa* (Gillespie & Soreng, 2005). This species is morphologically unusual in *Poa* and differs from all other *Poa* species (Hubbard, 1981) in having the styles terminal with no gap between them at the base, and stigmas that are elongated and simple with only short primary branches. In all other *Poa* species (Tzvelev, 1983; Watson & Dallwitz, 1992; Soreng, pers. obs.), the styles have a distinct gap between them at the base, and the stigmas are plumose with secondary branching. The species also differs from all *Poa* species in having apical cusps grading to short, stout apical awns. *Parodiochloa* differs from *Nicoraepoa* in its caespitose habit, elongated, deeply lacerate, glabrous ligules, and asymmetrically curved florets (Fig. 5S). Its adaxial leaf blade anatomy (Fig. 2E, F) is intermediate between that of *Poa* and *Nicoraepoa* in the ridge and valley adaxial epidermal topography, but the intercostal zones are covered by globose papillae, a condition unknown in *Poa* (except in five other sub-Antarctic island species; see next paragraph) and absent in *Nicoraepoa*. A few other species of *Poa* (e.g., *P. arida* Vasey and *P. keckii* Soreng) sometimes have single acentrically positioned papillae on some long cells of the abaxial leaf blade surfaces (Soreng, pers. obs.), a feature characteristic of *Puccinellia* Parl. and other genera of subtribe Puccinelliinae Soreng & J. I. Davis (Soreng et al., 2003b).

Five sub-Antarctic island species of *Poa* approach *Nicoraepoa* and *P. flabellata* in leaf blade anatomy and other characters. Edgar (1986), in a revision of New Zealand *Poa*, considered *P. cookii* and *P. ramosissima* Hook. f. to be a natural group that is likely allied to *P. flabellata*. *Poa flabellata* grows on oceanic islands adjacent to South America, *P. cookii*

Figure 4. A–G. *Nicoraepoa andina* (Trin.) Soreng & L. J. Gillespie. —A. Habit. —B. Inflorescence. —C. Ligule. —D. Spikelet. —E. Floret. —F. Anther (fertile). —G. Anther (sterile). (Drawn by S. G. Gómez. Reprinted from *Flora Patagónica* vol. 8(3): fig. 109, with permission from INTA.) H–O. *Nicoraepoa chonotica* (Phil.) Soreng & L. J. Gillespie. —H. Inflorescence. —I. Ligule. —J. Spikelet (perfect). —K. Floret (perfect). —L. Floret (pistillate). —M. Anther (fertile). —N. Anther (sterile) —O. Caryopsis. (Drawn by S. G. Gómez. Reprinted from *Flora Patagónica* vol. 8(3): fig. 113, with permission

on islands in the southern Indian Ocean, and *P. ramosissima* on islands south of New Zealand's South Island. *Poa cookii* shares many characteristics of *P. flabellata*. A close relationship between *P. flabellata* and *P. ramosissima* has now been demonstrated based on ITS sequence analyses (Gillespie et al., in prep.). Three additional New Zealand sub-Antarctic island species are considered closely related to *P. ramosissima* and *P. cookii*: *P. hamiltonii*, *P. foliosa*, and *P. tennantiana*. Edgar (1986) grouped the latter two species and treated *P. hamiltonii* as a synonym of *P. cookii*. We tentatively consider *P. hamiltonii* distinct from *P. cookii*, but agree there are few differences (see *Notes* in Taxonomic Treatment section under *P. hamiltonii*). This set of morphologically similar, southern-ocean island species has neither been united in an infrageneric classification nor formally grouped with *P. flabellata*. These five species are here placed in *Poa* sect. *Parodiochloa* with *P. flabellata*. Molecular relationships of the latter four species remain to be studied.

Several morphological features distinguish this newly expanded *Poa* sect. *Parodiochloa* (Table 2). The species are mostly large tufted grasses (except *P. ramosissima*), sometimes rhizomatous (*P. foliosa*, *P. tennantiana*), sometimes with pungent (*P. flabellata*) or semi-pungent leaf blades (*P. cookii*, *P. foliosa*, *P. hamiltonii*). Adaxial leaf blade surfaces are densely papillate on the costal or intercostal regions or both (all species, Figs. 2E, F, 3A–C; also noted by Edgar & Connor, 2000) (sometimes absent in *P. tennantiana*, Fig. 3D), and are otherwise mostly smooth (*P. ramosissima*, *P. tennantiana*) or quite scabrous (*P. cookii*, *P. flabellata*, *P. foliosa*, *P. hamiltonii*). The five to 42 lateral costal ridges (occasionally obscured by intercostal papillae) are tall (*P. cookii*) or have moderate or low adaxial epidermal relief in other species, and are one to three times wider than the intercostal valleys. The ligules are deeply and regularly lacerate (*P. cookii*, *P. hamiltonii*, *P. ramosissima*), elongate and entire to irregularly lacerate at the apex (*P. flabellata*), or short, truncate, and densely covered with short thick appressed hairs (*P. foliosa*, *P. tennantiana*). Panicle branches are mostly smooth but can be scabrid to hispid (some *P. cookii*). Lemma apices are generally slightly twisted and sharply pointed, with a short, somewhat thick attenuated cusp (*P. cookii*, *P. foliosa*, *P. hamiltonii*, *P. ramosissima*, *P. tennantiana*) grading to an awn up to 3 mm long in *P. flabellata*. The species are gynomonoecious (*P. cookii*, *P. hamiltonii*, *P. ramosissima*), dioecious (*P. foliosa*), or perfect-flowered (*P. tennantiana*) (Edgar, 1986; Edgar & Connor, 2000). Although *P. flabellata* was reported to be perfect-flowered (Anton & Connor, 1995), this needs further study. The stigmas are plumose and the styles are distinctly separate at the base, as is typical of Poeae, or the stigmas are slender and short-branched and the styles have no gap between them at the base (*P. flabellata*). The floret calluses may be glabrous (four species) or distinctly webbed (*P. foliosa* and most *P. ramosissima*). The glabrous state for calluses appears to be derived in this group, and thus the presence of a dorsal web is still an autapomorphy of *Poa*.

Key to the Genus *Nicoraepoa* and Selected Genera and Species of Subtribe Poinae

Genera of Poeae subtribe Poinae included in the key are: *Arctophila*, *Bellardiochloa*, *Dupontia*, *Festucella*, *Hookerochloa*, *Hyalopoa* (Tzvelev) Tzvelev, *Nicoraepoa*, and *Poa* (including sections *Austrofestuca*, *Madropoa*, p.p., *Parodiochloa*, *Siphonocoleus* Hitchc., and *Tzvelevia*). These genera share a perennial habit, multiple-flowered spikelets that are lanceolate in shape, and acute to acuminate glumes reaching over three fourths the length of the adjacent florets. Their caryopses have a non-linear, short hilum up to one third the length of the grain and are apically glabrous. The Poinae genus *Arctagrostis* is not included because it can be easily distinguished by its single-flowered spikelets. Likewise, genera of Alopecurinae and Miliinae may be distinguished by single-flowered spikelets. Genera of subtribe Puccinelliinae (Soreng et al., 2003b) similar to *Poa* in gross or overall morphology are excluded by their generally shorter glumes in combination with papillate leaf surfaces or single-flowered spikelets (*Catabrosa* P. Beauv., *Catabrosella* (Tzvelev) Tzvelev, *Oreopoa* H. Scholz & Parolly, *Paracolpodium* (Tzvelev) Tzvelev, *Phippsia* (Trin.) R. Br., *Pseudosclerochloa* Tzvelev, *Puccinellia*, *Sclerochloa* P. Beauv.).

Various combinations of characters can be used to discriminate among the above set of Poinae genera, but none holds up for all species. Thus, producing a key to the genera could be approached in a variety of ways depending on which characters are employed in which order. Leaf characteristics, including adaxial leaf blade surface morphology and ligule shape, seem to be the most consistently diagnostic for *Nicoraepoa*.

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from INTA.) P–S. *Nicoraepoa pugionifolia* (Speg.) Soreng & L. J. Gillespie. —P. Habit. —Q. Ligule. —R. Spikelet. —S. Floret. (Drawn by S. G. Gómez. Reprinted from *Flora Patagónica* vol. 8(3): fig. 112, with permission from INTA.)

Figure 5. A–D. *Nicoraepoa erinacea* (Speg.) Soreng & L. J. Gillespie. —A. Habit. —B. Ligule. —C. Spikelet. —D. Floret. (Drawn by I. Coloiera. Reprinted from *Flora Patagónica* vol. 8(3): fig. 110, with permission from INTA.) E–I. *Nicoraepoa robusta* (Steud.) Soreng & L. J. Gillespie. E. Habit. —F. Ligule. G. Spikelet. —H. Floret. —I. Palea. (Drawn by I. Coloiera. Reprinted from *Flora Patagónica* vol. 8(3): fig. 108, with permission from INTA.) J–N. *Nicoraepoa subenervis* (Hack.) Soreng & L. J. Gillespie subsp. *subenervis*. —J. Habit. —K. Ligule. —L. Spikelet. —M. Floret. —N. Palea. (Drawn by

1a. Adaxial leaf blade lateral surfaces prominently corrugated with multiple ridges and valleys, the ridges 2–5× wider than the valleys, without papillae; ligules 0.2–3 mm long, apices truncate (to slightly higher at the margins), margins entire or irregularly erose, never lacerate, ciliate or ciliolate (cilia 0.1–0.6 mm long); calluses glabrous or with a crown of straight to slightly curved hairs to 0.2–3 mm long, never webbed; plants usually of wet or seasonally wet, sometimes saline or subsaline habitats in southern South America . . . . . *Nicoraepoa*

1b. Adaxial leaf blade lateral surfaces flat or nearly flat or, if weakly to prominently corrugated with multiple ridges and valleys and the ridges up to 1–5× wider than the valleys, then the adaxial surface papillate in part (except some *Poa tennantiana*) and ligules commonly longer than 3 mm and sometimes deeply lacerate; ligules 0.2–25 mm long, apices truncate to acuminate, margins entire to deeply and regularly lacerate, smooth or infrequently asperous or ciliolate (cilia to 0.1 mm long); calluses glabrous or with a crown or dorsal tuft of hairs (webbed); plants of various habitats, not from South America (except *Nicoraepoa erinacea* and some *Poa*) 2

2a. Ligules deeply and more or less regularly lacerate 3

3a. Calluses with a crown of hairs 0.3–0.7 mm long; lemmas 3-nerved; adaxial leaf blade surfaces not papillate; plants of Northern Hemisphere high-latitude wetlands . . . . . *Arctophila*

3b. Calluses glabrous or webbed, infrequently with a few isolated hairs around the sides of the callus; lemmas 3- to 5-nerved 4

4a. Culms and nodes strongly compressed; sheaths fused to near their collars; adaxial leaf blade surfaces not papillate; plants of uplands in the Hawaiian Islands . . . . . *Poa* sect. *Siphonocoleus* . . . . . (*P. mannii* Munro, *P. sandvicensis* (Reichardt) Hitchc., and *P. siphonoglossa* Hack.)

4b. Culms and nodes terete; sheaths fused only in the lower 1/2; adaxial leaf blade surfaces densely papillate; plants of sub-Antarctic island coastal regions (*Poa* sect. *Parodiochloa*, p.p.) 5

5a. Culms slender, the vegetative portions prostrate, clambering on rocks; plants stooling, forming mats; leaf blades lax, soft, 1–2 mm wide, flat, abaxially and adaxially papillate; lemmas papillate . . . . . *Poa ramosissima*

5b. Culms stout, spreading to erect; plants caespitose, forming large dense tufts; leaf blades moderately firm, 2–10 mm wide, flat or folded, abaxially smooth, adaxially papillate in part; lemmas not papillate 6

6a. Fibrous ridges of basal sheaths smooth or irregularly sparsely scabrous; leaf blades adaxially nearly smooth or with scattered thick-based low hooks over the veins . . . . . *Poa hamiltonii*

6b. Fibrous ridges of basal sheaths regularly and closely pectinately scabrous (as in a sawfish's bill); leaf blades adaxially papillate and sometimes scabrous on the sides of the ridges with narrow-based spreading tipped hooks . . . . . *Poa cookii*

2b. Ligules entire or infrequently with few irregular, mostly shallow lacerations 7

7a. Adaxial leaf blade surfaces regularly corrugated with ridges 1–3× as wide as adjacent intercostal valleys . . 8

8a. Lemmas rounded to weakly keeled across the back, apex usually slender short-awned; leaf blades slender, 0.2–0.7(–1) mm wide, tightly involute; calluses with a crown of hairs often persisting at the tips of rachilla joints; panicle branches densely scabrous angled; caryopses semi-soft; alpine plants of southern Europe and the Middle East . . . . . *Bellardiochloa*

8b. Lemmas keeled across the back, apex cuspidate to stiffly short-awned; leaf blades broader, 2–10 mm wide, flat or folded, or the outer margins inrolled; calluses glabrous or webbed; panicle branches smooth or finely scabrous angled; caryopses firm; plants of sub-Antarctic islands and coasts of Tierra del Fuego, South America, and Shetland Islands (*Poa* sect. *Parodiochloa*, p.p.) 9

9a. Panicles tightly contracted; lemmas and calluses glabrous; ligules 6–23 mm long, abaxially smooth or sparsely scabrous . . . . . *Poa flabellata*

9b. Panicles contracted to loosely contracted; lemmas pubescent at least basally, calluses glabrous or webbed; ligules 1–16 mm long, abaxially densely scabrid to puberulent 10

10a. Ligules 1–3 mm; fibrous ridges of basal sheaths smooth; calluses webbed; panicles contracted . . . . . *Poa foliosa*

10b. Ligules 6–16 mm; fibrous ridges of basal sheaths scabrous; calluses glabrous; panicles loosely contracted . . . . . *Poa tennantiana*

7b. Adaxial leaf blade surfaces generally with only two central grooves, or with low ridges not more than 1× as wide as adjacent shallow valleys 11

11a. Calluses glabrous or with an isolated dorsal tuft of hairs; lemmas unawned . . . . . *Poa* (most species including section *Tzvelevia*)

11b. Calluses with a crown or beard of hairs around the base; lemmas awned or not 12

12a. Lemmas with a terminal or slightly subterminal awn; plants with coarsely fibrous old basal sheaths; panicles open, branches scabrous-angled; calluses with a crown or beard of short hairs to 1 mm long 13

13a. Leaf blades flat or folded, 2–7 mm wide, surfaces smooth or nearly so, margins scabrous . . . . . *Hookerochloa*

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I. Coloiera. Reprinted from *Flora Patagónica* vol. 8(3): fig. 107, with permission from INTA.) O S. *Poa flabellata* (Lam.) Raspail. —O. Habit. —P. Inflorescence. —Q. Ligule. —R. Spikelet. —S. Florets. (Drawn by F. Torres. Reprinted from *Flora Patagónica* vol. 8(3): fig. 88, with permission from INTA.)

13b. Leaf blades involute, 0.5–1.5 mm wide, surfaces and margins scabrous . . . . . *Festucella*

12b. Lemmas unawned; plants with or without fibrous old basal sheaths; panicles contracted or open, branches smooth or scabrous (rarely hispidulous), terete or angled; calluses with a crown or beard of short or longer hairs to 5 mm long 14

14a. Ligules short-truncate, to 1 mm long, margins ciliolate to ciliate; callus with a dense crown or beard of short hairs to 1 mm long; leaf blades ca. 1–2 mm wide, tightly folded with involute margins, firm to moderately firm, adaxially densely scabrid to coarsely hispidulous or soft-pubescent on and between the veins, abaxially smooth, glabrous; panicles contracted, dense or with few spikelets; stoloniferous to rhizomatous plants . . 15

15a. Panicles simple, sub-spicate, with fewer than 8 spikelets; plants forming low dense cushions to 25 cm tall; leaf blades up to 3(4) cm long, abaxially softly puberulent, stiff and sharply pungent; plants of interior sub-saline seasonally wet habitat in Patagonia, Argentina . . . . . *Nicoraepoa erinacea*

15b. Panicles branched, densely contracted, with more than 10 spikelets; plants dense to widely spreading, usually forming larger tufts, 20–100 cm tall; some or many leaf blades over 5 cm long, abaxially coarsely scabrous to stiffly puberulent, stiff and sharply pungent to moderately firm and weakly pungent; plants of coastal sands . . . 16

16a. Leaf blades firm, sharply pungent; flowers perfect; plants of Australia and New Zealand . . . . . *Poa billardierei* (sect. *Austrofestuca*)

16b. Leaf blades moderately firm, weakly pungent; flowers unisexual; plants of the Pacific coast of United States and Canada . . . . . *Poa* sect. *Madropoa*, p.p., *P. douglasii* Nees, and *P. macrantha*

14b. Ligules obtuse to acuminate, 1–18 mm long, margins glabrous; callus glabrous or with a short or longer crown of hairs to 5 mm long; leaf blades to 1.5–10 mm wide, flat, folded or involute, soft, adaxially smooth or scabrous mainly over the veins, abaxially smooth or scabrous, glabrous or infrequently stiffly puberulent; panicles contracted or open; plants of various habits 17

17a. Palea keels scabrid at least in part; panicle branches smooth or scabrous . . *Poa*, p.p.

17b. Palea keels smooth or nearly so; panicle branches smooth 18

18a. Lemmas distinctly keeled; callus with a diffuse crown of fine soft weakly woolly hairs 1–2.5 mm long . . . . . *Hyalopoa*

18b. Lemmas weakly keeled; callus with a tuft of straight or sinuous hairs on the surrounding lemma base 19

19a. Leaf blade abaxial surface strongly ribbed, flat; glumes usually much shorter than adjacent florets; paleae subequal to lemmas; florets 2 to 6; lemmas glabrous, apices obtuse . . . . . *Arctophila*

19b. Leaf blade abaxial surface smooth or weakly ribbed, flat or weakly involute; glumes subequal or longer than adjacent florets; florets 1 to 2; paleae distinctly shorter than lemmas; lemmas glabrous or softly pubescent, apices acute to acuminate . . . . . *Dupontia*

Taxonomic Treatment

Nicoraepoa Soreng & L. J. Gillespie, gen. et nom. et stat. nov. Replaced name: *Poa* subgen. *Andinae* Nicora, Hickenia 1(18): 99. 1977. TYPE: *Nicoraepoa andina* (Trin.) Soreng & L. J. Gillespie.

Perennials; gynodioecious to weakly dioecious, or synecious; stoutly rhizomatous, rhizomes 1–3 mm diam., short or long; major roots 1–2 mm diam.; vegetative branching intra- and extra-vaginal; sheaths of basal leaves becoming coarsely fibrous in age, smooth or sparsely scabrous between veins; sheaths of upper culm leaves open to near base or margins joined by a narrow hyaline membrane up to 1/4 the sheath in length; ligules 0.2–3 mm long, thickly membranous to chartaceous, abaxially smooth or pubescent with appressed to loose hairs, apices truncate or longest near the margins, irregularly erose ciliolate to ciliate (with hairs to 0.5 mm long); blades firm (fairly soft in *N. subenervis*), flat, folded or involute, 2–15 mm wide, abaxially smooth or the keel scabrid near apices, glabrous, adaxially with (2)4 to 48 deep to moderately deep intercostal valleys that are smooth or more often scabrous, separated with blocky flat-topped or broadly rounded costal ridges (anatomically with adaxial epidermis underlain by sclerenchyma caps or with "T"- or "I"-shaped girders extending to the abaxial epidermis in cross section), 1–5× broader than the valleys, without papillae, smooth or commonly scabrous especially on the sides of the ridges, glabrous or rarely short stiff puberulent (*N. erinacea*), apices pungent (weakly so in *N. subenervis*). Panicle axis and branches smooth or less often scabrous, pedicels smooth or scabrous, glabrous (or rarely coarsely hispidulous). Spikelets 2- to 5-flowered; rachilla internodes terete, usually scabrous; glumes subequal, smooth or keels scabrous, chartaceous throughout or apically scarious, 1- or 3-veined, lateral veins faint or distinct, subequal to the adjacent lemmas; calluses blunt, glabrous or with a crown of 0.2–3 mm long, straight or slightly curved hairs;

lemmas keeled, apices symmetrical and not twisted, acutely pointed, mucronate, or awned from a sinus up to 0.2 mm deep, glabrous or with stiff hairs on the keel to 1 mm long and sometimes on the marginal veins at the base, smooth throughout or scabrous over much of the surface, weakly veined, awnless or with a slender, erect, briefly divergent or weakly sinuous, slender, scabrid, 0.2–3(5) mm long awn from the keel vein (without lateral merging veins); palea keels scabrous or coarsely ciliate; lodicules 2, 1–2 mm long, membranous, lanceolate, apically entire or irregularly short-toothed; anthers 3, 2–3 mm long, staminodes in pistillate flowers 1–3 mm long. Caryopsis glabrous, firm or less often semi-soft, lipid present; hilum round to narrowly elliptical, 1/5–1/3 the grain in length; style bases terminal, with a gap between them, stigmas plumose, white.

Distribution and habitat. Argentina and Chile, from moderately high elevations in the Andes at about 32°S in the province of San Juan, Argentina, and Region V, Chile, south through Patagonia to coastal and interior regions of Tierra del Fuego, Isla de Estados, and Falkland/Malvinas Islands (Nicora, 1978; Moore, 1983). Perennially or seasonally wet ground, or in shallow water, in fresh water or frequently in subsaline, saline, or mineralized soils of coastal sands and estuaries, springs, bogs, marshes, springy meadows, wet rocks, and riparian meadows, from sea level to over 3200 m.

Notes. The new genus name commemorates the late Elisa G. Nicora, Argentine specialist in *Poa*, author of *Poa* subg. *Andinae*, and influential, longtime student of South American Poaceae who passed away in 2001 (Rúgolo de Agrasar, 2001). The subgenus epithet *Andinae* is a plural adjective and cannot be satisfactorily employed as a plural Latinized noun. Also, there is a genus *Andinia* (Luer) Luer in Orchidaceae. *Nicoraepoa* has six species and two subspecies. A good key to the species is available in Nicora (1978).

1. **Nicoraepoa andina** (Trin.) Soreng & L. J. Gillespie, comb. nov. Basionym: *Poa andina* Trin., Linnaea 10(3): 306. 1836. TYPE: Chile. [Region Bío-Bío, province Bío-Bío]: "austr. in alp. frigid. mont. igniv. Antuco," [1829], *E. Pöppig* [*D.-907*] (holotype, LE [TRIN-2578.01a]!; isotypes, LE [TRIN-2578.01a] fragment at BAA-2450b!, LE!). Figure 4A–G.

Poa acrochaeta Hack., Repert. Spec. Nov. Regni Veg. 10(243–247): 172. 1911. TYPE: Chile. [Region Maule, province Curicó]: Vulkan Peteroa, s.d., *comm. K. F. Reiche s.n.* [collector questionable: *448* in Philippi script on label (possibly *M. Vidal Gormaz* in 1891 according to E. Nicora annotation)] (holotype, SGO [PHIL-448] W 39455!; isotypes, SGO [PHIL-448] fragment and photo at US 1723707!, W 39455 fragment at US 88711!).

Poa aristata Phil., Anales Univ. Chile 43: 574. 1873. TYPE: Chile. [Region Los Lagos, province Osorno]: Volcán de Osorno, [1872], *don Carlos Juliet s.n.* (holotype, SGO [PHIL-439] W 39450!; isotypes, BAA-2457!, SGO [PHIL-439] fragment and photo at US 89700!).

Deschampsia latifolia Phil., Linnaea 29(1): 91. 1858, non *Deschampsia latifolia* Hochst. ex A. Rich., 1850. TYPE: Chile. [Region Maule, province Linares]: in Andibus dep. [Cordillera] Linares, *Germain s.n.* (holotype, SGO [PHIL-197] W not seen; isotypes, SGO 63483!, SGO [PHIL-197] fragment and photo at US 556494!).

Distribution and habitat. Argentina (Neuquén), Chile (Araucanía, Bío-Bío, Los Lagos, Maule), in the central Patagonian Andes (Nicora, 1978). Stream banks and wet meadow at high elevations.

Notes. Many SGO Philippi types of Poaceae were loaned to US ca. 1904 (where fragments were retained and specimens photographed; see U.S. National Herbarium Type Specimen Register, <http://ravenel.si.edu/botany/types/>), returned to SGO, and then sent to Edward Hackel in Vienna, where they may be seen today, generally with W accession numbers on the SGO sheets.

Additional specimen examined. CHILE. **Araucanía:** Laguna Captrén, *R. J. & N. L. Soreng 7193* (CONC, US).

2. **Nicoraepoa chonotica** (Phil.) Soreng & L. J. Gillespie, comb. nov. Basionym: *Poa chonotica* Phil., Linnaea 29(1): 97. 1858. TYPE: Chile. [Region Aysén, province Aysén]: [Chonos-Islands], "in monte Cerro de Chonos ad circa 1290 ped. s.m.," *Dr. Fonk s.n.* (holotype, SGO [PHIL-410] W *s.n.*!; isotypes, SGO [PHIL-410] fragment at BAA!, SGO [PHIL-410] fragment and photo at US 89683!). Figure 4H–O.

Poa berningeri Pilg., Notizbl. Bot. Gart. Berlin-Dahlem 10(97): 761. 1929. TYPE: Chile. [Region Los Lagos], province Llanquihue: Volcán Yates, ca. 1200 m, "vereinzelt zwischen *Nothofagus* an der Waldgrenze," Mar. 1925, *Werdermann 1698* (holotype, B not seen; isotypes, B fragment at BAA!, B fragment at US 89693! [pistillate]).

Poa borchersii Phil., Anales Univ. Chile 94: 172. 1896. TYPE: Chile. [Region Bío-Bío, province Ñuble]: "prope thermas chillanenses," [Baños de Chillan, Jan. 1883], *Augustus Borchers s.n.* (holotype, SGO [PHIL-431] W 39457!; isotypes, US fragment at BAA!, SGO [PHIL-431] fragment and photo US 89691a!, W 29457 fragment at US 89691b!).

Poa chubutensis Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 7: 196. 1902. TYPE. Argentina. Province Chubut: [region Río Corcovado, 43°S, 71°W], "in rupestribus collinis prope Teka-choique, [1–15 Feb.] aest. 1901," *N. Illin* [*90*] (holotype, LP!; isotypes, BAA 2518!, SI!, US 916986!, LP [SPEG] US 1503958!).

Poa latifolia Phil., Linnaea 29(1): 97. 1858, non *Poa latifolia* G. Forst., Fl. Ins. Austr. 4, no. 44. 1786. TYPE: Chile.

[Region Aysén, province Aysén]: [Chonos-Islands], "in monte Cerro de Chonos ad circa 1290 ped. s.m.," *Dr. Fonk s.n.* (holotype, SGO [PHIL-414] W 39453!; isotypes, SGO fragment at BAA-2613!, SGO fragment at BAA-2612!, SGO 63493!, SGO [PHIL-414] fragment and photo at US 88768!).

Poa robusta Phil., Anales Univ. Chile 43: 574. 1873, non *Poa robusta* Steud., 1854. TYPE: Chile. [Region Los Lagos, province Llanquihue]: Volcán de Calbuco, [1872], *Carlos Juliet s.n.* (holotype, SGO [PHIL-443] W 39419!; isotype, SGO [PHIL-443] fragment and photo at US 88733!).

Distribution and habitat. Argentina (Chubut, Neuquén, Río Negro), Chile (Araucanía, Aysén, Los Lagos, Magallanes), in central and southern Patagonian Andes and coastal reaches of southwestern Chile. Low to upland marshes, riverbanks, and wet rocks.

Notes. This species was called *Poa borchersii* (Nicora, 1978), but Soreng et al. (2003a) determined that the older name *P. chonotica* applies to the species. Viviparous specimens of this species are common but can be separated from *Nicoraepoa robusta* by their looser or open panicles, shorter glumes, and frequent presence of a few hairs around the callus.

Additional specimens examined. CHILE. **Araucanía:** Lonquimay, *A. Pfister 7949* (CONC). **Aysén:** Seno Ventisquero, *R. J. & N. L. Soreng 7254* (CONC, US). **Magallanes:** Última Esperanza, Río Pingo, *R. J. & N. L. Soreng 7349* (CONC, US).

3. Nicoraepoa erinacea (Speg.) Soreng & L. J. Gillespie, comb. nov. Basionym: *Poa erinacea* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 7: 198. 1902. TYPE: Argentina. [Province Chubut]: "in aridissimis subsalsis secus Río Chubut," Dec. 1899, *N. Illin s.n.* (holotype, LP 67!; isotypes, LP BAA 2534!, LP fragment at US 88783!). Figure 5A–D.

Distribution and habitat. Argentina (Chubut), in arid parts of Patagonia (Nicora, 1978). Subsaline springs, at mid-elevations.

Notes. This is one of the odder species of the new genus, as the leaf blades are narrow and softly hairy, with few intercostal valleys on the adaxial surface. However, as in other *Nicoraepoa* species, the callus has a distinct crown of hairs, the short stiff leaf blades are apically pungent, and the ligules are densely ciliate.

4. Nicoraepoa pugionifolia (Speg.) Soreng & L. J. Gillespie, comb. nov. Basionym: *Poa pugionifolia* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 7: 199. 1902. TYPE: Argentina. [Province Santa Cruz]: "Párr-aik (Río Sehuen), Feb 1898," *C. Ameghino s.n.* (lectotype, designated here, LP [SPEG-65]!); isotype, [LP-SPEG-65 fragment] BAA-2672!. Figure 4P–S.

Poa acutissima Pilg., Repert. Spec. Nov. Regni Veg. 12: 306. 1913. TYPE: Argentina. Süd-Patagonien, [1907–1908], *C. Skottsberg s.n.* (holotype, B not seen; isotypes, BAA 2442!, BAA 2443!, B fragment at US 88712!).

Distribution and habitat. Argentina (Santa Cruz, Tierra del Fuego), Chile (Magallanes), in Patagonia. Low- to mid-elevation subsaline meadows and open ground in semi-arid Patagonia.

Notes. LP-SPEG-65 is unambiguous as a lectotype. The two original specimens in Speggazini's herbarium are taxonomically indistinguishable, but the place, river, and month recorded on the labels are arranged differently than in the original protologue. The protologue stated, "in rupestribus porphyricis Párr-aik secus Río Chico et Boron-aik secus Río Sehuen, Jan. et Feb. 1898, *C. Ameghino s.n.*" The protologue has the localities and rivers and dates switched, as on a map we located Párr-aik south of the Río Sehuen, which is well south of the Río Chico. This is in agreement with the chosen lectotype and its isotype BAA-2672, "Párr-aik (Rio Sehuen)," which gives the date as February, "2-1898." We have not been able to locate Boron-aik on a map, but the syntype at LP [SPEG-66] says Boron-aik (Rio Chico), Jan. 1898.

Additional specimens examined. ARGENTINA. **Santa Cruz:** Güer Aike, 9 km S of Río Gallegos, *P. M. Peterson et al. 17062* (SI, US); Buenos Aires, *P. M. Peterson et al. 17254* (SI, US). CHILE. **Magallanes:** Última Esperanza, Sierra Baguales, *R. J. & N. L. Soreng 7336* (CONC, US).

5. Nicoraepoa robusta (Steud.) Soreng & L. J. Gillespie, comb. nov. Basionym: *Poa robusta* Steud., Syn. Pl. Glumac. 1: 426. 1854. TYPE: Chile. [Region] Magallanes, [province Magallanes]: "Punta Arenas, paludibus salsis lit. maris prope, Dec.," *W. Lechler* [*Hohenacker exsiccatae*] *1191* (holotype, P [STEUD] not seen; isotypes, G fragment at BAA!, P fragment at BAA!, G not seen, LE!, K!, W, K, P fragments at US 81586!, LE fragment at US 946984!, W not seen). Figure 5E–I.

Festuca arenaria Lam., Encycl. 1: 191. 1791, non *Festuca arenaria* Osbeck, 1788. *Poa arenicola* St.-Yves, Candollea 3: 282. 1927, non *Poa arenaria* Lam., Tabl. Encycl. 1: 183. 1771. TYPE: [Chile. Region Magallanes, either province Magallanes or Tierra del Fuego]: E. Region of Magellan, in arenis maritimis, *M. Commerson s.n.* (holotype, P [LAM] not seen; isotypes, MPU not seen, P [LAM] fragment at US 2875377b!, MPU fragment and photo at US 2875377a! [perfect-flowered]).

Distribution and habitat. Argentina (Santa Cruz, Tierra del Fuego), Chile (Magallanes), in southern

Patagonia, Tierra del Fuego, Isla de los Estados, and Falkland/Malvinas Islands. Coastal wet meadows and beaches.

Notes. In this species there are normal-flowered and viviparous forms. The normal-flowered forms always have glabrous lemmas and calluses. In the viviparous forms, these structures may be glabrous, or, more commonly, there are some short sparse hairs on the callus. The latter plants are separable from *Nicoraepoa chonotica* viviparous plants by their narrowly lanceolate panicles and long glumes. Reports from farther north in Chile, Aysén Region, represent viviparous plants of *N. chonotica*.

6. Nicoraepoa subenervis (Hack.) Soreng & L. J. Gillespie, comb. nov. Basionym: *Poa subenervis* Hack., Ark. Bot. 7(2): 7, t. 2, f. 3. t. 7, f. 1908. TYPE: Argentina. [Province Santa Cruz]: Patagonia, "in paludosis inter, Lago Viedma et Laguna Tar, ca. 1000 m, 27 Feb. 1905," *P. K. H. Dusén 6021* (lectotype, designated here, W not seen; isotypes, K fragment at BAA 2704-1!, US fragment at BAA 2704-2!, W fragment at US 88723!).

Distribution and habitat. Argentina, Chile, in Patagonia. Upland vegas and subsaline springs in arid mountains.

Notes. The protologue reads: "Argentina. E. & S. Patagonia: 'in montanis inter lacum Lago Viedma et lacum Laguna Tar ad marginem paludis in alt c 1000 m; ad flumen Río Fosiles in montanis uliginosis c 800 m,' *Dusén.*" Three specimens agree with the type protologue: (1) "inter lacum Lago Viedma et lacum Laguna Tar," *P. K. H. Dusén 5927* (syntype, comm. J. F. Mullins in 1922 BAA 2703!); (2) "Lago San Martin, Río Fosiles in uliginosis c. 800 m, 1 'A.' 1905," *P. K. H. Dusén* (syntype, S not seen); and (3) *P. K. H. Dusén 6021* (cited above as the lectotype). *P. K. H. Dusén 6021* was selected as lectotype because duplicates and fragments are present in several herbaria.

6a. Nicoraepoa subenervis subsp. **subenervis**. Basionym: *Poa subenervis* Hack. var. *subenervis*.

Distribution and habitat. Argentina (Santa Cruz), Chile (Magallanes) (Nicora, 1978). Southern Patagonia. Figure 5J–N.

6b. Nicoraepoa subenervis subsp. **spegazziniana** (Nicora) Soreng & L. J. Gillespie, comb. nov. Basionym: *Poa subenervis* var. *spegazziniana* Nicora, Hickenia (18): 103. 1977. *Poa spegazziniana* Parodi, Dansk Bot. Ark. 22(1): 67. 1963, nom. nud. TYPE: Argentina. Province Mendoza: Dpto. San Rafael, Atuel Valle, a Arroyo Colorado [lat. 34°, 2200 m, 19 Nov. 1955], *T. W. Böcher, Hjerting & K. Rahn 1306* (holotype, BAA 2706-1!).

Distribution and habitat. Argentina (Mendoza, Neuquén [Nicora, 1978], and San Juan), Chile, (Libertador General Bernardo O'Higgins, Metropolitana), in the northern Patagonian Andes.

Additional specimens examined. ARGENTINA. **San Juan:** Río del Agua Negra, *P. M. Peterson et al. 19296* (SI, US). CHILE. **Libertador General Bernardo O'Higgins:** Sewell, Río Coya, *Jiles s.n.* (CONC 134734). **Metropolitana:** above Embalse del Río Yeso, *R. J. & N. L. Soreng 7155* (CONC, US).

SIX SUB-ANTARCTIC ISLAND *POA* SPECIES ARE PLACED IN *POA* SECT. *PARODIOCHLOA*

Poa sect. **Parodiochloa** (C. E. Hubb.) Soreng, Contr. U.S. Natl. Herb. 48: 579. 2003. Basionym: *Parodiochloa* C. E. Hubb., Bull. Brit. Mus. (Nat. Hist.), Bot. 8: 395. 1981, non *Parodiochloa* A. M. Molina, 1986. TYPE: *Festuca flabellata* Lam. (≡ *Poa flabellata* (Lam.) Hook. f.).

Notes. The section as here emended has six species.

1. Poa cookii (Hook. f.) Hook. f., Philos. Trans. 168: 22. 1879. Basionym: *Festuca cookii* Hook. f., Fl. Antarct. 2: 382, t. 139. 1846. TYPE: [France. Kerguelen Islands]: Kerguelen's Land, Christmas Harbour, on rocks and in moist places always near the sea, abundant, May–Aug. 1840, *J. D. Hooker 762* (lectotype, designated by Edgar, 1986, New Zealand J. Bot. 24: 433, K [H2003/00969-290!]; isotype, LE not seen).

Distribution and habitat. Sub-Antarctic islands of the southern Indian Ocean: Crozet Islands, Heard Island, Kerguelen Islands, Marion Island, and Prince Edward Islands (Edgar, 1986, without voucher citations). Edgar's (1986) records for *Poa cookii* s.l. on Macquarie Island are referred to *P. hamiltonii*. Specimens reported by the Australian Antarctic Data Center (2007) from Crozet Islands (e.g., Possession Islands, *W. A. Proctor 00257* [BM not seen]) and Heard Island (e.g., *J. M. R. Hughes & R. D. Seppelt 24056* [ADT not seen]) are presumed to be *P. cookii* s. str. based on Edgar's comments about differences in *P. cookii* s.l. plants from Macquarie Island from the rest of the species. Coastal wet rocks and bluffs, and wet slopes, to 100 m, often associated with penguin colonies. The Australian Antarctic Data Center (2007) reported *P. cookii* from Gough Island based on *E. W. Groves* collections, but these (and other specimens

seen by us) were verified by C. E. Hubbard (Groves, 1981) as *P. flabellata*. Two other specimens were cited in Hooker's original protologue: (1) Hab. Kerguelen's Land, *Anderson* (in Cook's Voyage) *s.n.* (syntype, not seen), and (2) Kerguelen's Land, *D. Lyall s.n.* (syntype, K [H2003/00969-289]!).

Additional specimens examined. SOUTH AFRICA. **Prince Edward Islands:** Marion Island, *S. Fugler 125* (US); Prince Edward Island, *B. J. Huntly 975* (K).

2. Poa flabellata (Lam.) Raspail, Ann. Sci. Observ. 2: 86. t. 4. f. 11. 1829. Basionym: *Festuca flabellata* Lam., Encycl. 2: 462. 1788. *Parodiochloa flabellata* (Lam.) C. E. Hubb., Bull. Brit. Mus. (Nat. Hist.), Bot. 8: 396. 1981. TYPE: [Chile. Region Magallanes, province Magallanes or Tierra del Fuego]: Cette plante a été trové au détroit de Magellan, [Dec. 1767–Jan 1768], *M. Commerson s.n.* (holotype, P [LAM] not seen; isotype, P [LAM] two fragments at US 2875414!). Figure 50–S.

Dactylis caespitosa G. Forst., Fasc. Pl. Magell. 12. 1788. *Festuca antarctica* Spreng., Pl. Min. Cogn. Pug. 2: 23. 1815. *Festuca caespitosa* (G. Forst.) Roem. & Schult., Syst. Veg. 2: 732. 1817, non *Festuca caespitosa* Desf., 1798. *Poa forsteri* Steud., Syn. Pl. Glumac. 1: 260. 1854. *Poa caespitosa* (G. Forst.) Hook. ex Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 5: 91. 1896, non *Poa caespitosa* Poir., 1804. TYPE: [Argentina. Province Tierra del Fuego]: [Isla de los Estados], Novi Anni insulis, s.d., *Forster s.n.* (types, BM not seen, K!, KIEL not seen, LE [TRIN-2791.2]!, UPS [T-2347] not seen, K [FORST] fragment at US 2851271!, W not seen).

Festuca urvilleana Steud., Syn. Pl. Glumac. 1: 312. 1854. TYPE: [Falkland/Malvinas Islands.] Ins. Maclov., "sec. specimen ex Herbo. *Urville* sub *F. flabellata* Lam.," *d'Urville s.n.* (holotype, P [URVILLE] not seen).

Poa controversa Steud., Syn. Pl. Glumac. 1: 260. 1854. *Poa controversa* var. *minor* Steud., Syn. Pl. Glumac. 1: 260. 1854. TYPE: Falkland Islands. Port William, "sub *Dactylis caespitosa*," [4 Sep. 1850], *W. Lechler* [(*Hohenacker exsiccata*) *106*] (holotype, P [STEUD-385] not seen; isotype, P [STEUD] fragment at BAA not seen, P [STEUD-385] fragment at US 2851272!).

Sesleria americana Nees ex Steud., Syn. Pl. Glumac. 1: 296. 1854. TYPE: Argentina. [Province Tierra del Fuego]: [Isla de los Estados], Staten Island, Sep. [1829], *Chanticleer* [the ship], [*W. H. B. Webster s.n.*] (holotype, CGE [LINDL] not seen).

Distribution and habitat. South Atlantic Ocean and Drake Passage to the Pacific Ocean, mainly or always on islands. Argentina (Isla de los Estados, SE Tierra del Fuego), Chile (Tierra del Fuego, Isla Diego Ramirez), Falkland Islands/Islas Malvinas, South Georgia Island, Tristan da Cunha (Gough Island) (Nicora, 1978; Groves, 1981; Moore, 1983). It was introduced and established in the Shetland Islands (Edmondson, 1980; Groves, 1981) and was probably introduced to Gough Island (Groves, 1981). Coastal wet rocks, bluffs, and wet rocky slopes to 100 m, often associated with penguin colonies.

Notes. Reported from mainland Chile along the north side of the Straits of Magellan based on the *Commerson* type collection for *Festuca flabellata*. Moore (1983) indicates Commerson's collections from the Tierra del Fuego area were all from along the north shore of the Straits of Magellan and the adjacent Isla Isabella, in Chile. It is impossible to plot this collection precisely; moreover, the species has not been collected in this area since. Possibly the original location given on the *Commerson* label was in error. Only four other collection localities for the species from west of Isla de los Estados are known to us (three cited above, and one cited by Nicora, 1978).

Additional specimens examined. ARGENTINA. **Tierra del Fuego:** [SE end of Isla Grande], Policarpo, *R. N. P. Goodall 2272* (BAB, NA, SI). CHILE. **Magallanes:** Cape Horn, Hermit Island, *J. D. Hooker 68* (K); Isla Diego Ramírez, *E. E. Pisano-Valdés 3405* (CONC). UNITED KINGDOM. **South Georgia Island:** *J. Smith M-1148* (K). **Tristan da Cunha Islands:** Gough Island, *N. M. Wasce G207* (K).

3. Poa foliosa (Hook. f.) Hook. f., Handb. N. Zeal. Fl. 338. 1864. Basionym: *Festuca foliosa* Hook. f., Fl. Antarct. 1: 99, t. 55. 1844 [1845]. TYPE: [New Zealand.] Lord Auckland's Islands: abundant, especially in rocky places near the sea, on the ground forming large green tufts on the cliffs never far from the sea, Dec. 1840, Antart. Exp. 1839–1843, *J. D. Hooker s.n.* (holotype, K [H203/00969-288]!; isotypes, fragment CHR 278601 not seen, LE not seen).

Distribution and habitat. New Zealand. Sub-Antarctic Islands: islets around Stewart Island, Solander Islands, Antipodes Islands, Auckland Islands, Campbell Island, and Macquarie Island (Edgar, 1986). Coastal wet rocks, bluffs, and adjacent wet slopes.

Notes. *Poa foliosa* is similar to *P. ramosissima* in its leaf blade architecture, as the adaxial valleys are very shallow between the costal ridges and are covered by papillate long cells. It is presumed to be closely related to *P. cookii*, which also has papillate long cells between its veins. *Poa foliosa* has a web on the callus of the lemma, whereas *P. cookii* does not. Chromosome number: $2n = 28$ (Edgar & Connor, 2000).

4. Poa hamiltonii Kirk, Trans. & Proc. New Zealand Inst. 27: 353. 1895. TYPE: New Zealand. Sub-Antarctic Islands, Macquarie Island, 1891, *Hamilton s.n.* (holotype, K [H2003/00969–295]!; isotype, WELT 66728 not seen).

Distribution and habitat. New Zealand sub-Antarctic Islands: Macquarie Island (Edgar, 1986). Coastal wet rocks, bluffs, and adjacent wet slopes.

Notes. There are two collections at Kew with the same data. The holotype, K [H2003/00969-295], is a spindly plant that has the lacerate ligules of *Poa cookii*, and keys to this species, but in some respects approaches *P. ramosissima*. This sheet has "*Poa Hamiltonii n.s.*" [in Kirk's handwriting?] and a diagnosis. A second sheet with the same collection information, K [H2003/00969-336]!, has only the determination "*Poa foliosa*," and is that species.

Edgar (1986) placed *Poa hamiltonii* in *P. cookii* but noted that, in the Macquarie Island plants, the panicles are overtopped by the leaves and are somewhat narrower, and spikelets are on average slightly shorter. From the specimens on the four sheets we have seen from Macquarie Island, in addition to the differences noted by Edgar (1986), it appears that *P. hamiltonii*, unlike *P. cookii*, lacks prickle hairs on the sheaths and adaxial surfaces of the leaf blades. Thus, until we have reviewed other collections from Macquarie Island, we prefer to maintain this as a separate species closely related to *P. cookii*.

5. Poa ramosissima Hook. f., Flora Antarctica 1(1): 101. 1844. [1845.] TYPE: [New Zealand]. Hab. Lord Auckland's Islands, "very common on the rocks overhanging the sea, trailing over banks, &c.," [hangs down from the cliffs and rocks near the sea, common, very stoloniferous, Nov. 1840], *J. D. Hooker s.n.* (holotype, K [H2003/00969–298]!).

Distribution and habitat. New Zealand. Sub-Antarctic Islands, Auckland Islands, and Campbell Island (Edgar, 1986). Wet rocks and bluffs by the coast.

Notes. The specimens seen entirely lack prickle hairs, and both surfaces of the leaf blades are covered in papillate long cells. Papillate long cells are an anatomical feature characteristic of the adaxial blade surfaces of *Poa cookii*, *P. foliosa*, and *P. hamiltonii* but are not always expressed in *P. tennantiana*, and only weakly so in material examined of *P. flabellata*. Hooker identified two pubescence variations in the florets of *P. ramosissima*—one with a web on the callus and one without—but most material from both islands is webbed (Edgar, 1986). ITS DNA data place this species with *P. flabellata* (Gillespie & Soreng, unpublished data). Chromosome number: $2n = 28$ (Hair, 1968).

6. Poa tennantiana Petrie, Subantarctic Is. N. Z. 2: 467. 1909. *Poa foliosa* var. *tennantiana* (Petrie) Cheeseman, Man. N. Z. Fl. (ed. 2) 188. 1925. TYPE: New Zealand. The Snares, 9 Jan. 1890, *T. Kirk* (lectotype, designated by Edgar, 1986, New Zealand J. Bot. 24: 188, WELT 36063 not seen).

Distribution and habitat. New Zealand South Island and New Zealand sub-Antarctic Islands: islets off Stewart Island, Snares Islands, Antipodes Islands, and Auckland Islands (Edgar, 1986). Coastal wet rocks and adjacent slopes.

Notes. *Poa tennantiana*, unlike *P. cookii*, *P. hamiltonii*, *P. foliosa*, and *P. ramosissima*, sometimes lacks papillae on the long cells of the leaf blade surfaces. (Edgar [1986] noted the adaxial leaf blade surfaces of this species were minutely papillose, but papillae were not seen in the material we examined.) Like the first two species, but unlike the latter two, it has glabrous calluses. It also has moderately deep narrow valleys and broad blocky ridges on leaf blade adaxial surfaces and pectinately scabrous sheaths as in *P. cookii* and *P. flabellata*. Chromosome number: $2n = 28$ (Hair, 1968).

TZVELEVIA REDUCED TO A SECTION IN *POA*

Poa sect. **Tzvelevia** (E. B. Alexeev) Soreng & L. J. Gillespie, comb. et stat. nov. Basionym: *Tzvelevia* E. B. Alexeev, Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol. 90(5): 103. 1985. TYPE: *Poa kerguelensis* (Hook. f.) Steud.

Notes. Monotypic.

Poa kerguelensis (Hook. f.) Steud., Syn. Pl. Glumac. 1: 257. 1854. Basionym: *Triodia kerguelensis* Hook. f., Fl. Antarct. 379, t. 138. 1847. *Festuca kerguelensis* (Hook. f.) Hook. f., Philos. Trans. 168: 22. 1879. *Tzvelevia kerguelensis* (Hook. f.) E. B. Alexeev, Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol. 90(5): 103. 1985. TYPE: [France. Kerguelen Islands]: Kerguelen's land, "rocky places, at 300–1200 ft." [on the debris of the rocks common "perennial" and alpine, Christmas Harbour, May 1840], *J. D. Hooker 761* (holotype, K [H2003/00969–293]!; isotypes, CN not seen, CN CGE fragment at US 1127149!).

Distribution and habitat. Sub-Antarctic Islands of Indian Ocean: Heard Island and Kerguelen Islands. Wet rocky upland to alpine slopes. Reported also from Heard Island (*J. M. R. Hughes & R. D. Seppelt 24053* [ADT not seen]; fide Australian Antarctic Data Center, 2007).

Notes. *Poa kerguelensis* was placed in its own genus, *Tzvelevia*, principally based on its having a long narrow hilum (Alexeev, 1985). The hilum seen in US material is actually between one fifth and one fourth the length of the fruit and narrowly ovoid, just at the long end of hilum lengths in species of *Poa*. We return it to *Poa*, as there is nothing else to distinguish the genus by, but note that it needs further study as to its position in the genus.

POA SECT. *PLICATAE* FROM ARGENTINA AND CHILE IS PLACED IN *POA* SECT. *ACUTIFOLIAE*

Poa sect. **Acutifoliae** Pilg. ex Potztal, Willdenowia 5(3): 473. 1969. TYPE: *Poa acutifolia* Hauman [= *P. planifolia* Kuntze].

Poa sect. *Plicatae* Pilg. ex Potztal, Willdenowia 5(3): 472. 1969. TYPE: *Poa plicata* Hack.

Distribution and habitat. Northwestern Patagonia from the province of Neuquén, Argentina, and adjacent valleys of the Andes of Chile (Coquimbo, Metropolitana, and Valparaíso), and Argentina north to the province of Salta, Argentina. *Poa acinaciphylla* (syn. *Poa villaroelii* Phil., fide Nicora, 1978; Soreng et al., 2003a): Argentina (Mendoza), Chile (Coquimbo, Metropolitana, and Valparaíso). *Poa planifolia*: Argentina (Mendoza), Chile (Metropolitana). *Poa plicata*: Argentina (Catamarca, La Rioja, Salta, and Tucumán; Negritto & Anton, 2000). *Poa stepparia*: Argentina (Neuquén; Nicora, 1978). Alpine stream margins and wet meadows and steppe, sometimes in subsaline conditions.

Notes. *Poa plicata*, the sole species of section *Plicatae*, is here formally placed in section *Acutifoliae*, which now contains four species: *P. acinaciphylla*, *P. planifolia*, *P. plicata*, and *P. stepparia*. The species have pungent leaf apices but otherwise have ligule margins and leaf blade surface anatomy typical of *Poa* (except for *P. stepparia*). Callus hairs, when present, arise from the dorsal side of the lemma as is typical for *Poa* (callus hairs in *P. stepparia* are not clearly distinct from the keel and marginal vein hairs), and the lemma surfaces are glabrous (except for hairs at the base of the keel and marginal veins in *P. stepparia*). The placement of *P. stepparia* needs further investigation.

Additional specimens examined. *Poa acinaciphylla*: ARGENTINA. **Mendoza:** Laguna de Solsneado, *P. M. Peterson & C. R. Annable 11380* (US). CHILE. **Coquimbo:** Cordillera de Combarbala, Potrero Grande, *Jiles 4829* (CONC). **Metropolitana:** Río Yerba Loca, *R. J. & N. L. Soreng 7169* (CONC, US). **Valparaíso:** Laguna Castro, *Zoellner 932* (CONC).

Poa planifolia: CHILE. **Metropolitanas:** above Embalse del Río Yeso, *R. J. & N. L. Soreng 7160* (CONC, US).

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Erratum

Livshultz, T., D. J. Middleton, M. E. Endress & J. K. Williams. 2007. Phylogeny of Apocynoideae and the APSA clade (Apocynaceae s.l.). Ann. Missouri Bot. Gard. 94: 324–359.

Livshultz et al. (2007) miscoded *Carissa spinarum* L. as having sinistrorse corolla aestivation (character 3, state 0) and misidentified its geographical distribution as restricted to Africa (Fig. 1 in Livshultz et al., 2007). Species of *Carissa* have either sinistrorse or dextrorse corollas (Leeuwenberg, 2001). *Carissa spinarum* has a dextrorse corolla (character 3, state 1) and is widely distributed in Africa, Asia, and Australia (Leeuwenberg, 2001).

The correct coding of *Carissa spinarum* renders the evolution of corolla aestivation ambiguous when mapped onto the phylogenetic tree presented in Figure 1 of Livshultz et al. (2007). It is equally parsimonious (two steps) to reconstruct a dextrorse corolla as evolving independently in *C. spinarum* and in the most recent common ancestor of the APSA clade minus Wrighteae or as a synapomorphy for *C. spinarum* plus the APSA clade with a reversal to a sinistrorse corolla in Wrighteae. The phylogeny presented by Simões et al. (2007), which sampled *C. ovata* R. Br. (syn. *C. spinarum*, dextrorse corolla) and *C. macrocarpa* (Eckl.) A. DC. (sinistrorse corolla), indicates that independent evolution of dextrorse aestivation in *C. spinarum* on the one hand and the APSA clade minus the Wrightieae on the other, as in Livshultz et al. (2007), is the more parsimonious interpretation when *Carissa* is better sampled.

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Cover illustration. *Ainsliaea aptera* DC., drawn by Susana Freire and inked by Hugo Calvetti.